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TOXIC HAZARDS RESEARCH UNIT ANNUAL TECHNICAL REPORT: 1980

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TECHNICAL REVIEW AND APPROVAL

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals, "Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER



ANTHONY A. THOMAS, MD
Director
Toxic Hazards Division
Air Force Aerospace Medical Research Laboratory

ERRATA SHEET

AFAMRL-TR-80-79

TOXIC HAZARDS RESEARCH UNIT ANNUAL TECHNICAL REPORT: 1980

Pages 105, 106, and 107

The word Fyrquel was misspelled a total of six times on these three pages. Delete the spelling Fryquel, insert Fyrquel.

Page 106. - The table appearing on this page should appear as follows:

<u>Compound</u>	<u>Numbers Showing Response 24 hrs</u>	<u>Response 48 hrs</u>	<u>Sensitizing Potential</u>	<u>Mean Reaction Score (24 hrs)</u>	<u>Sensitizing Response</u>
WGF-200D (accumulator)	13	13	Severe	93	Moderate
WGF-200D (barrel)	14	11	Severe	80	Moderate
Houghto-Safe 271	2	4	Slight	34	Mild
Houghto-Safe 273	0	0	None	--	None
Fyrquel-220	4*	5*	None	39*	None

* Values recorded were the result of mild irritation from the peanut oil vehicle and were also seen in the vehicle control injection areas of the same animals.

Page 107. - 3rd paragraph after the heading "Determination of the Dermal Toxicity of JP-10", first line, 7th word, delete "mg/kg". Replace with "gm/kg".

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19. KEY WORDS (Continue on reverse side if necessary and identify by block number) <table> <tbody> <tr> <td>Methylhydrazine</td> <td>Jet Fuels</td> <td>RJ-5</td> </tr> <tr> <td>Hydrazine</td> <td>JP-5</td> <td>Shale Oil</td> </tr> <tr> <td>1,2-Dimethylhydrazine</td> <td>JP-4</td> <td>Diesel Fuel</td> </tr> <tr> <td>1,1-Dimethylhydrazine</td> <td>JP-10</td> <td>Emergency Exposure Limit (Cont'd)</td> </tr> </tbody> </table>			Methylhydrazine	Jet Fuels	RJ-5	Hydrazine	JP-5	Shale Oil	1,2-Dimethylhydrazine	JP-4	Diesel Fuel	1,1-Dimethylhydrazine	JP-10	Emergency Exposure Limit (Cont'd)
Methylhydrazine	Jet Fuels	RJ-5												
Hydrazine	JP-5	Shale Oil												
1,2-Dimethylhydrazine	JP-4	Diesel Fuel												
1,1-Dimethylhydrazine	JP-10	Emergency Exposure Limit (Cont'd)												
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The research programs of the Toxic Hazards Research Unit (THRU) for the period of June 1979 through May 1980 are reviewed in this report. Chronic toxicity or oncogenic studies were carried out with methylcyclohexane, tricyclodecane, purified 1,1-dimethylhydrazine and bicycloheptadiene. A subchronic inhalation study was conducted with shale derived JP-5 and DFM fuels. Acute toxicity studies were conducted on a variety of chemical agents used by the Air Force and Navy.														

BLOCK 19.

Antifouling Paints
Methylcyclohexane
Bicyclopentadiene
Toxicity
Carcinogenesis
Oncogenesis
Hydraulic Fluids
Fluomine
Irritation
Skin
Percutaneous
Oral
Inhalation
Sensitization
Metabolites
Dermal

PREFACE

This is the seventeenth annual report of the Toxic Hazards Research Unit (THRU) and concerns work performed by the Department of Community and Environmental Medicine of the University of California, Irvine on behalf of the Air Force under Contract Number F33615-76-C-5005. This document constitutes the fifth report under the current contract and describes the accomplishments of the THRU from June 1979 through May 1980.

The current contract for operation of the Laboratory was initiated in 1975 under Project 6302, "Occupational and Environmental Toxic Hazards in Air Force Operations," Task 01, "Toxicology of Propellants and Materials," Work Unit Number 63020115. K. C. Back, Ph.D., Chief of the Toxicology Branch, was the technical contract monitor for the Air Force Aerospace Medical Research Laboratory.

This is a co-sponsored U. S. Air Force/U. S. Navy research effort. That portion of the work effort sponsored by the U. S. Navy was under the direction of LCDR Morris J. Cowan, Jr., MSC, USN, and identified as Work Unit Number MF58524025.4012 "Toxicity Evaluation and Validation of Exposure Guidelines for Use in Naval Operational Environments".

J. D. MacEwen, Ph.D., served as Laboratory Director for the THRU of the University of California, Irvine and as co-principal investigator with T. T. Crocker, M.D., Professor and Chairman, Department of Community and Environmental Medicine. Acknowledgement is made to A. K. Roychowdhury, Ph. D., C. E. Johnson, C. C. Haun, and G. L. Fogle for their significant contributions and assistance in the preparation of this report. Partial support for this program was provided by the U. S. Naval Medical Research Institute and the Department of Transportation.

TABLE OF CONTENTS

<u>Section</u>	<u>Page</u>
I INTRODUCTION	13
II RESEARCH PROGRAM	14
A Study of the Oncogenic Potential of Inhaled Monomethylhydrazine	14
A Study of the Oncogenic Potential of Inhaled Hydrazine after Chronic Low Level Exposure	16
A Study of the Oncogenic Potential of Purified UDMH in Mice	26
1,2-Dimethylhydrazine Dihydrochloride as a Positive Tumorigenic Control for the Experimental Animal Species Used in Current Oncogenic Studies	27
The Evaluation of the Oncogenic Potential of Inhaled Hydrazine in Rats and Hamsters After a Short Series of Weekly One-Hour Exposures	31
Evaluation of the Toxic Effect of a 90-Day Continuous Inhalation Exposure to Petroleum JP-4 Vapor	32
Evaluation of the Toxic Effect of a 90-Day Continuous Exposure to Petroleum JP-5 Vapor	46
A Subchronic Inhalation Toxicity Study of a 90-Day Continuous Inhalation Exposure to Shale JP-5/Aviation Gas Mixture	51
A Subchronic Toxicity Study of a 90-Day Con- tinuous Inhalation Exposure to Shale JP-5 Vapor	54
A Subchronic Toxicity Study of a 90-Day Continuous Inhalation Exposure to Petroleum Diesel Fuel Marine	60
A Subchronic Toxicity Study of a 90-Day Con- tinuous Inhalation Exposure to Shale Diesel Fuel Marine	65

TABLE OF CONTENTS (CONT'D)

<u>Section</u>	<u>Page</u>
A Subchronic Toxicity Study of a 90-Day Continuous Inhalation Exposure to Decalin	72
The Experimental Determination of the Oncogenic Effects of Petroleum JP-4 Vapor	78
The Experimental Determination of Safe Atmospheric Exposure Concentrations of JP-10 Vapor	80
A 12-Month Chronic Inhalation Exposure of Animals to Methylcyclohexane to Determine Its Oncogenic Potential	83
The Experimental Determination of Safe Atmospheric Exposure Concentrations for RJ-5	90
Chronic Effects of Low Level Inhalation Exposures to Fluomine Particulates	95
Emergency Exposure Limit Studies With JP-10	101
Evaluation of the Irritation Potential of Several Hydraulic Fluids	104
Determination of the Dermal Toxicity of JP-10	107
Determination of the Acute Toxicity Potential of Navy Antifouling Paint Formulations	107
Percutaneous, Oral and Inhalation Studies For Classification of Toxicity Ratings For Transportable Chemical Agents	112
III	
FACILITIES	116
Computer Program Development	116
Gas Chromatographic Evaluation of RJ-5	117
Quality Control Analysis for Fyrquel-220	119
Evaluation of Shale JP-5 Contamination with Aviation Gasoline	121

TABLE OF CONTENTS (CONT'D)

<u>Section</u>	<u>Page</u>
Physiological Fluids - Determination of Methylcyclohexane Metabolites by Gas Chromatography/Mass Spectrometry	125
Chamber Technician Training Program	127
Animal Technician Training Program	127
REFERENCES	131

LIST OF FIGURES

<u>Figures</u>	<u>Page</u>
1. Effect of inhaled UDMH on mouse body weight	28
2. Mortality of mice exposed to UDMH for 1 year	28
3. JP-4 vapor introduction system	35
4. Alarm and emergency shut down system for hydrocarbon fuels vaporization	36
5. Inhalation chamber analysis system for hydrocarbon fuels	37
6. Effect of 90-Day continuous inhalation exposure to petroleum JP-4 vapor on serum globulin of beagle dog blood	39
7. Effect of 90-day continuous inhalation exposure to petroleum JP-4 vapor on serum total protein of beagle dog blood	40
8. Effect of 90-day continuous inhalation exposure to petroleum JP-4 vapor on blood urea nitrogen (BUN) in beagle dogs	41
9. Effect of 90-day continuous inhalation exposure to petroleum JP-4 vapor on rat weight	44
10. Effect of 90-day continuous inhalation exposure to petroleum JP-5 vapor on male rat body weight	47
11. Effect of 90-day continuous inhalation exposure to petroleum JP-5 vapor on female rat body weight	48
12. Effect of 90-day continuous inhalation exposure to shale JP-5 vapor on male rat body weight	57
13. Effect of 90-day continuous inhalation exposure to shale JP-5 vapor on female rat body weight	58
14. Effect of 90-day continuous exposure to petroleum DFM vapor on male rat body weight	61
15. Effect of 90-day continuous exposure to petroleum DFM vapor on female rat body weight	62

LIST OF FIGURES (CONT'D)

<u>Figures</u>		<u>Page</u>
16.	Effect of 90-day continuous inhalation exposure to shale DFM vapor on male rat body weight	68
17.	Effect of 90-day continuous inhalation exposure to shale DFM vapor on female rat body weight	69
18.	Effect of 90-day continuous inhalation exposure to decalin vapor on male rat body weight	73
19.	Effect of 90-day continuous inhalation exposure to decalin vapor on female rat body weight	74
20.	Mean body weights of rats and hamsters exposed intermittently to 100 ppm JP-10 for one year	82
21.	Effect of methylcyclohexane exposure on Fischer 344 rat body weight	87
22.	Effect of inhalation exposure to methylcyclohexane in golden syrian hamster body weight	88
23.	Mean body weights of rats and hamsters exposed intermittently to RJ-5 vapor	94
24.	Effect of fluomine dust inhalation on male rat body weight	99
25.	Effect of fluomine dust inhalation on guinea pig body weight	100
26.	Comparison of gas chromatograms of RJ-5 run at the THRU and commercial supplier	118
27.	Gas chromatogram of Fyrquel-220	120
28.	Gas chromatogram of aviation gasoline (grade 100/130)	122
29.	Gas chromatogram of liquid fuel used in chamber exposures contaminant	123
30.	Gas chromatogram of uncontaminated liquid JP-5 shale fuel	123

LIST OF FIGURES (CONT'D)

<u>Figures</u>		<u>Page</u>
31.	Gas chromatogram of 3% aviation gasoline (grade 100/130) in uncontaminated shale oil JP-5	124
32.	Partial gas chromatograms of control and MCH exposed rat urine with and without 50 ppm each of the isomeric methylcyclohexanol added	126

LIST OF TABLES

<u>Table</u>		<u>Page</u>
1.	Pathologic Effects of 1-Year Inhalation Exposures To Monomethylhydrazine on Golden Syrian Hamsters	17
2.	Experimental Design For Hydrazine Inhalation Exposure Concentrations	17
3.	Incidence of Histologic Lesions in Beagle Dogs Exposed to Inhaled Hydrazine and Their Unexposed Controls	19
4.	Selected Tumors Found in Female Fischer 344 Rats After Inhalation Exposure to Hydrazine	21
5.	Selected Tumors Found in Male Fischer 344 Rats After Inhalation Exposure to Hydrazine	22
6.	Pathologic Changes Seen in Female Fischer 344 Rats After Inhalation Exposure to Hydrazine	23
7.	Pathologic Changes Seen in Male Fischer 344 Rats After Inhalation Exposure to Hydrazine	24
8.	Neoplastic Pathology in Control and Hydrazine Exposed Female C57B1/6 Mice	25
9.	Mortality Response to Weekly Subcutaneous Injections of SDMH Dihydrochloride	30
10.	Incidence of Histologic Lesions in Golden Syrian Hamsters After Weekly Injection with SDMH and Their Unexposed Controls	30
11.	Clinical Hematology and Chemistry Tests Performed on Dogs and Rats Exposed to Petroleum JP-4 Vapor	38
12.	Effect of 90-Day Continuous Exposure to Petroleum JP-4 Vapor on Red Blood Cell Fragility in Beagle Dogs	42
13.	Effect of 90-Day Continuous Exposure to Petroleum JP-4 Vapor on Organ Weights	43
14.	Mean Hematology and Clinical Chemistry Values of Male Rat Immediately After 90-Day Continuous Exposure to Petroleum JP-4 Vapor	45

LIST OF TABLES (CONT'D)

<u>Table</u>		<u>Page</u>
15.	Mean Hematology and Clinical Chemistry Values of Female Rats Obtained Immediately After 90-Day Continuous Exposure to Petroleum JP-4 Vapor	45
16.	Mean Hematology and Clinical Chemistry Values of Male Rats 19 Months After 90-Day Continuous Exposure to Petroleum JP-5 Vapor	49
17.	Mean Hematology and Clinical Chemistry Values of Female Rats 19 Months After 90-Day Continuous Exposure to Petroleum JP-5 Vapor	50
18.	Male Rat Organ Weights Measured 19 Months After 90-Day Continuous Exposure to Petroleum JP-5	50
19.	Female Rat Organ Weights Measured 19 Months After 90-Day Continuous Exposure to Petroleum JP-5	51
20.	Effect of 90-Day Continuous Exposure to Shale JP-5/Aviation Gas on Dog Red Blood Cell Fragility	53
21.	Effect of 60-Day Continuous Exposure to Shale JP-5/Aviation Gas on Organ Weights of Beagle Dogs	54
22.	Effect of 60-Day Continuous Exposure to Shale JP-5 Vapor on Red Blood Cell Fragility in Beagle Dogs	55
23.	Effect of 90-Day Continuous Exposure to Shale JP-5 Vapor on Organ Weights of Beagle Dogs	56
24.	Hematology and Clinical Chemistry Values of Male Rats After 90-Day Continuous Inhalation Exposure to Shale JP-5 Vapor	59
25.	Hematology and Clinical Chemistry Values of Female Rats After 90-Day Continuous Inhalation Exposure to Shale JP-5 Vapor	59
26.	Effect of 90-Day Continuous Exposure to Shale JP-5 on Organ Weights of Male Rats	60

LIST OF TABLES (CONT'D)

<u>Table</u>		<u>Page</u>
27.	Effect of 90-Day Continuous Exposure to Shale JP-5 on Organ Weights of Female Rats	60
28.	Hematology and Clinical Chemistry Values of Male Rats 19 Months After 90-Day Continuous Inhalation Exposure to Petroleum DFM Vapor	63
29.	Hematology and Clinical Chemistry Values of Female Rats 19 Months After 90-Day Continuous Inhalation Exposure to Petroleum DFM Vapor	63
30.	Mean Organ Weights of Male Rats 19 Months After 90-Day Continuous Exposure to Petroleum DFM Vapor	64
31.	Mean Organ Weights of Female Rats 19 Months After 90-Day Continuous Exposure to Petroleum DFM Vapor	64
32.	Effect of 90-Day Continuous Exposure to Shale DFM Vapor on Red Blood Cell Fragility of Beagle Dogs	66
33.	Effect of 90-Day Continuous Exposure to Shale DFM Vapor on Organ Weights of Beagle Dogs	67
34.	Hematology and Clinical Chemistry Values of Male Rats After 90-Day Continuous Inhalation Exposure to Shale DFM Vapor	70
35.	Hematology and Clinical Chemistry Values of Female Rats After 90-Day Continuous Inhalation Exposure to Shale DFM Vapor	71
36.	Effect of 90-Day Continuous Exposure to Shale DFM Vapor on Organ Weights of Male Rats	71
37.	Effect of 90-Day Continuous Exposure to Shale DFM Vapor on Organ Weights of Female Rats	72
38.	Hematology and Clinical Chemistry Values of Male Rats Obtained 19 Months After 90-Day Continuous Exposure to Decalin Vapor	75
39.	Hematology and Clinical Chemistry Values of Female Rats Obtained 19 Months After 90-Day Continuous Exposure to Decalin Vapor	75

LIST OF TABLES (CONT'D)

<u>Table</u>		<u>Page</u>
40.	Organ Weights of Male Rats 19 Months After 90-Day Continuous Exposure to Decalin Vapor	76
41.	Organ Weights of Female Rats 19 Months After 90-Day Continuous Exposure to Decalin Vapor	76
42.	Clinical Hematology and Chemistry Tests Performed on Rats Exposed to Petroleum JP-4 Vapor	80
43.	Mortality Ratios for Groups of JP-10 Exposed and Control Animals at Exposure Conclusion and 10 Months Postexposure	81
44.	JP-10 Exposure Mean Monthly Concentrations Presented to Experimental Animals	83
45.	Mean Methycyclohexane Concentration Measured in Animal Exposure Chambers	86
46.	Rat Organ Weights Measured at the Conclusion of One-Year Exposure to Methycyclohexane	89
47.	Mean Hematology and Clinical Chemistry Values of Male Rats After A One-Year Inhalation Exposure to MCH Vapor	90
48.	Mean Hematology Values of Female Rats After A One-Year Exposure to MCH Vapor	90
49.	Animal Distribution in RJ-5 Exposure Chambers	92
50.	Significant Histopathology Findings in Dogs Immediately After 6-Month Exposure to Fluomine Aerosols	96
51.	Incidence of Chronic Inflammation of Trachea in Rats Immediately Following Exposure to Fluomine Dust	96
52.	Effect of 6-Month Inhalation Exposure to Fluomine Dust on Rat Lung Weight	97
53.	Bronchopneumonia in Rats Exposed to Fluomine Dust	97
54.	Alveolar/Bronchiolar Carcinomas in Mice Exposed to Fluomine Dust	98

LIST OF TABLES (CONT'D)

<u>Table</u>		<u>Page</u>
55.	Alveolar/Bronchiolar Adenomas in Mice Exposed to Fluomine Dust	98
56.	Rabbit Eye Irritation Results After Application of Antifouling Paint Formulations	110
57.	List of Compounds Submitted by the U.S. Department of Transportation for Acute Inhalation, Peroral and Percutaneous Toxicity Studies	113
58.	Oral Toxicity of Various Compounds to Male and Female Rats	114
59.	Dermal Toxicity of Various Compounds to Female Rabbits	114
60.	One-Hour Inhalation Toxicity of Various Compounds for Male and Female Rats	114
61.	Corrosive Effect of Pentachlorophenol on Rabbit Skin	115
62.	Gas Chromatographic Conditions for Separation of RJ-5	117
63.	Comparison of Relative Peak Areas in Gas Chromatograms of RJ-5	119
64.	Gas Chromatographic Conditions for Fyrquel-220 Analysis	119

SECTION I

INTRODUCTION

This document constitutes the 17th annual report of the Toxic Hazards Research Unit (THRU), a research team which operates a dedicated inhalation toxicology laboratory to investigate potentially hazardous chemicals and materials of interest to the U. S. Air Force, U. S. Navy, and other governmental agencies. The THRU research team is an interdisciplinary group of University of California, Irvine, toxicologists, chemists, statisticians, and engineers supported by Air Force pathologists, veterinarians, and medical technologists.

The research facilities used by the THRU consist of animal exposure chambers and supporting laboratories which have previously been described by MacEwen (1965), Fairchild (1967), and Thomas (1968).

During the first six years of operation, the primary research efforts of the THRU were directed to obtaining information on health hazards of spacecraft flight, and the biological data obtained have been used as criteria for setting continuous exposure limits and for engineering design factors. The primary research efforts have in recent years focused more on problems of aircraft environments, chronic occupational health problems, and the potential oncogenicity of chemicals used in military and civilian activities. To this end, many of the current research programs serve the mutual interests of the U. S. Air Force, U. S. Navy, and other governmental agencies.

As part of its contractual responsibilities, UCI/THRU presents an annual technical conference to disseminate new toxicological information to the U. S. Air Force and other governmental and industrial scientists. This year's conference was chaired by Anna Baetjer, Sc.D., Professor Emeritus, Johns Hopkins School of Hygiene and Public Health. Twenty-one technical papers covering a broad range of occupational and environmental toxicology problems and a two-hour refresher course in basic immunology were presented. Seven papers were presented by University of California faculty and staff members. The open forum discussions following each session resulted in significant contributions of additional technical information and scientific exchange. The conference, held 13 November through 15 November 1979, drew 140 participants including speakers.

The papers presented at the conference were prepared for publication as the Proceedings of the 10th Conference on Environmental Toxicology which is a separate technical report (AFAMRL-TR-79-121).

Our next conference, currently in the development stage, will be held in November 1980 at the Imperial House South Motel, Dayton, Ohio.

SECTION II

RESEARCH PROGRAM

The research activity of the THRU is a continuing program independent of contract years, with several studies in progress at the beginning and end of each report period. Experiments that were initiated and completed during the past year and were of sufficient magnitude to merit separate technical reports are only summarized in this document. This year's research program was conducted on a broad range of chemical materials and includes inhalation studies of rocket and aircraft fuels. Acute oral and dermal toxicity studies on a variety of materials were also conducted.

A STUDY OF THE ONCOGENIC POTENTIAL OF INHALED MONOMETHYLHYDRAZINE

Hydrazines administered in the drinking water of Swiss mice and Golden Syrian hamsters have been reported by Toth (1972, 1973) to have carcinogenic activity. In the first of these studies, solutions of 0.001% methylhydrazine sulfate were given daily ad libitum to 5 and 6 week old randomly bred Swiss mice for their entire lifetimes. Hydrazine and methylhydrazine sulfate significantly increased incidence of lung tumors in the mice, while methylhydrazine enhanced the development of neoplasms by shortening the latent period. In the second of Toth's studies, Golden Syrian hamsters received 0.01% methylhydrazine in drinking water daily ad libitum for life. Malignant histiocytomas (Kupffer cell sarcomas) were observed in the livers of 54% of the male hamsters treated, while none were observed in the control groups.

Earlier studies of MMH carcinogenicity by Kelly et al. (1969) and Roe et al. (1967) did not demonstrate any increase in tumor incidence over control animals. Roe administered 0.5 mg MMH per day by mouth to Swiss mice on a 5 day/week for 40 weeks schedule and found a lower incidence of tumor bearing mice (pulmonary adenomas) compared to untreated controls. Kelly reported per os administration of 0.2 ml MMH solution/mouse to female CDF₁ mice, and i.p. administration of 0.1 ml MMH solution/mouse in male mice of the same strain produced no more lung adenomas or leukemias than were found in untreated controls after 8 weeks of treatment. The MMH was given as a 2% aqueous solution.

MacEwen and Vernot (1975) reported the results of a two-year drinking water study in which hamsters were given untreated and acidified drinking water (pH 3.5) containing 0.01% MMH. A third group of hamsters was given acidified water as unexposed controls. Neither the incidence, degree of severity, nor age of onset of nonneoplastic pathologic changes was markedly different between animals drinking MMH in water and control animals. The presence of 23% incidence of adrenocortical tumors in untreated

control animals versus 4% in the group given MMH in tap water and 12% in the hamsters receiving MMH in acidified water argues against MMH as a cause of these tumors. The remaining neoplasms, one hemangioendothelioma of the liver, two hepatocellular carcinomas, one cutaneous melanoma, occurred only in the experimental groups. They were derived from four different cell types and as such constitute a 4% incidence for each tumor in their respective groups of animals, except for an 8% incidence of hepatocellular carcinoma. The overall tumor incidence for the hamsters receiving MMH + tap water was 16%, for those treated with MMH in acidified water was 24%, and for the unexposed control was 31%. These findings are in contrast to the findings of Toth and Shimizu (1973).

The reported investigations presented some evidence that MMH may be carcinogenic and therefore may pose a hazard to men. The case for carcinogenicity of MMH was, however, inconclusive at this point and for this reason, the comprehensive inhalation exposure study described herein was undertaken.

Rats, mice, hamsters, and dogs were exposed to MMH by the inhalation route in chambers for one year using an industrial work week schedule of 6 hours/day, 5 days/week with holidays and weekends off to simulate an industrial exposure regimen for man.

All rodents were held for an additional year of observation at which time necropsies were performed on survivors and approximately 33 tissues were taken for histopathologic evaluation of tumorigenesis following the National Cancer Institute protocol. The dogs will continue to be held for additional postexposure observation. They are located at the vivarium at Wright-Patterson Air Force Base, Ohio.

Previous annual reports (MacEwen and Vernot, 1977, 1978, 1979) contain experimental data including mortality, body weight measurements, and clinical chemistry results of dogs tested during the 12 months of MMH exposure and through 14 months post-exposure. The dogs will continue to be held and examined until March 1982.

An incident occurred on 8 June 1979 with a dog exposed to 2.0 ppm MMH. During routine postexposure physical examinations, this dog, as well as all others, was dosed with a tranquilizer (acepromazine) prior to examination and teeth cleaning. This dog had an apparent reaction to the drug (the dog had previously received this drug with no effect) which resulted in a temperature increase to 108F. Cold water baths and a cold water enema reduced the temperature to normal. The dog experienced convulsions and refused to drink water. During the two-week period following the incident, the dog was lethargic and unresponsive. After two weeks, the dog slowly resumed normal behavior and at 18 days appeared to be 80% recovered. Blood sampled two weeks after the reaction showed high SGPT (290) and alkaline phosphatase

(66). A blood sample two weeks later showed these parameters back to normal; SGPT - 4; alkaline phosphatase - 9.4. However, it was felt that the value of this dog had been compromised and evaluation at a later date would be impossible. This dog and a comparable control were therefore sacrificed on 12 July 1979.

The body weights of the remaining MMH exposed dogs are comparable to their respective controls two years postexposure, and blood parameters have continued within normal ranges during the same period.

Histologic examination of rat and mouse tissues is presently being done and will be reported in the next annual report. Examinations on hamster tissues have been completed and are currently being subjected to statistical analysis. Some of the findings that appear to be related to MMH exposure are shown in Table 1. There appears to be a generalized and possible dose related reduction in amyloidosis with exposure to MMH in liver, spleen and kidney. There are also dose related decreases in lymphoid hyperplasia of the lymph nodes. An increase in biliary cysts and hepatitis incidence is noted in exposed animals. There are also small but dose related pulmonary changes but most noticeable are the effects on the upper respiratory system. Exposure to MMH increased the incidence of submucosal cysts, rhinitis and hyperplasia and at 2.0 ppm and 5.0 ppm exposure levels produced nasal polyps. In a comparison of malignant versus benign tumors there were 28 malignant and 21 benign tumors in 45 unexposed control hamsters and 20 malignant and 50 benign tumors in 60 hamsters exposed to 5 ppm MMH for one year.

A STUDY OF THE ONCOGENIC POTENTIAL OF INHALED HYDRAZINE AFTER CHRONIC LOW LEVEL EXPOSURE

Partial results of a study conducted to determine the chronic effects of inhaled hydrazine on rats, mice, hamsters, and dogs and the oncogenic potential of hydrazine in rodents placed in long-term observation after one year of industrial-type inhalation exposure (MacEwen et al., 1979) were presented at the 10th Conference on Environmental Toxicology.

The annual report of 1975 (MacEwen and Vernot, 1975) provided the complete details of the experimental protocol while subsequent annual reports contain a progressive accumulation of experimental data including mortality, body weight measurements, and clinical chemistry results for dogs tested during the 12 months of hydrazine exposure and through the postexposure phase of the study.

This report provides a brief sketch of the design of the study, pathology results for dogs that were not available for presentation at the 1979 conference, and a summation of results.

A total of 3900 rodents and 24 dogs were used in the hydrazine study which spanned a time period of approximately 4 years. The animal groups consisted of C57B1/6 mice, Fischer 344 rats, Golden Syrian hamsters, and beagle dogs. The number of animals of each species and sex are shown in Table 2 which also lists the chambers used and exposure concentrations.

TABLE 1. PATHOLOGIC EFFECTS OF 1-YEAR INHALATION EXPOSURES TO MONOMETHYLHYDRAZINE ON GOLDEN SYRIAN HAMSTERS (INCIDENCE RATIO)

TISSUE	UNEXPOSED CONTROLS	0.2 PPM EXPOSED	2.0 PPM EXPOSED	5.0 PPM EXPOSED
<u>Liver</u>				
Amyloidosis	38/194	31/175	23/177	24/174
Hepatitis	20/194	15/175	24/177	31/174
Biliary Cysts	41/194	67/175	73/177	76/174
<u>Spleen</u>				
Amyloidosis	34/168	20/148	18/158	19/159
<u>Lymph Nodes</u>				
Reticuloendothelial Tumors	7/192	5/167	2/170	6/168
Lymphoid Hyperplasia	26/192	12/167	15/170	6/168
<u>Kidney</u>				
Amyloidosis	60/195	48/179	37/176	38/177
Interstitial Fibrosis	75/195	83/179	105/176	96/177
<u>Adrenals</u>				
Cortical Adenoma (Benign)	16/191	16/173	10/172	23/176
Cortical Adenoma (Malignant)	11/191	14/173	11/172	10/176
<u>Lung</u>				
Atelectasis	0/189	2/177	5/174	7/174
Inter-Alveolar Histiocytosis	8/189	8/177	5/174	15/174
<u>Nares/Trachea/Bronchi</u>				
Submucosal Cysts	35/190	52/177	56/180	46/177
Rhinitis	12/190	21/177	25/180	28/177
Hyperplasia	0/190	0/177	2/180	4/177
Adenoma	1/190	0/177	0/180	7/177
Polyps	0/190	0/177	9/180	11/177
<u>Bone</u>				
Osteoma	0/190	0/180	2/181	1/182

TABLE 2. EXPERIMENTAL DESIGN FOR HYDRAZINE INHALATION EXPOSURE CONCENTRATIONS

Hydrazine Concentration (ppm)	Animal Numbers, Sex, and Species	Chamber Number
0.05	100♂, 100♀ rats; 400♀ mice	7
0.25	200♂ Hamsters; 400♀ mice	5
0.25	100♂, 100♀ rats; 4♂, 4♀ dogs	6
1.0	200♂ hamsters; 400♀ mice	1
1.0	100♂, 100♀ rats, 4♂, 4♀ dogs	4
5.0	100♂, 100♀ rats; 200♂ hamsters	8
Control	150♂, 150♀ rats; 800♀ mice, 200♂ hamsters; 4♂, 4♀ dogs	Vivarium

The exposure concentrations were selected to span the range from the current OSHA Threshold Limit Value for exposure to hydrazine (1 ppm) and the proposed ACGIH Threshold Limit Value of 0.1 ppm. The 5 ppm exposure concentration was selected as a maximum tolerable exposure dose which would produce some biological response without causing death in hamsters and rats. Mice and dogs were not exposed at this concentration because prior studies (Haun and Kinkead, 1973) had shown that repeated daily exposures to 5 ppm hydrazine caused death in these species.

An unexposed control dog died 32 months postexposure due to respiratory failure following extensive hemorrhage into the thoracic cavity. The hemorrhage resulted from rupture of numerous small capillaries formed in response to pyogranulomatous reaction involving the lung, pericardium, and diaphragm. Bacterial cultures made from the material in this lesion isolated a Corynebacterium organism.

BSP (Bromsulfalein) retention time was measured in all dogs at bimonthly intervals during the study. There was no indication that liver function was affected by exposure to either 0.25 or 1.0 ppm hydrazine exposure daily for the one-year period. However, during the postexposure phase of the study, commencing at 34 months postexposure, one dog from the 1 ppm hydrazine exposure group exhibited intermittent increases in SGPT values. BSP retention time measured when these increases occurred was never greater than that of control animals nor was the liver palpable on examination. After multiple episodes of this cyclic event, the animal was sacrificed at 36 months postexposure and tissues examined. A control dog was also sacrificed for comparative pathology. Changes in the liver of the exposed dog were revealed as groups of swollen cells that had water clear cytoplasm. The change was seen with multifocal distribution.

The surviving 21 dogs were sacrificed at 38 months post-exposure (50 months on study). Incidence of histopathologic lesions seen in various tissues of all the dogs exposed to 0.25 ppm or 1.0 ppm hydrazine and controls is given in Table 3.

Only 2 tumors were seen, both in one dog exposed to 0.25 ppm hydrazine. There was a hemangioma of endothelial cells of the splenic capsule. Also seen was a low grade papillary carcinoma of epithelial tissue at the mucocutaneous border of the anus. Four months postexposure, a rectal tumor had been detected in this dog which prompted a biopsy of a growth on the surface of the rectum. Histologic examination revealed a low grade adenocarcinoma. This diagnosis was confirmed at 27 months postexposure when the tumor was removed and examined. It appears from the tumor pathology in Table 3 that surgical removal of the tumor 11 months prior to sacrifice was incomplete.

TABLE 3. INCIDENCE OF HISTOLOGIC LESIONS IN BEAGLE
DOGS EXPOSED TO INHALED HYDRAZINE AND
THEIR UNEXPOSED CONTROLS N = 8

<u>LESION TYPE</u>	<u>1.0 PPM EXPOSURE GROUP</u>	<u>0.25 PPM EXPOSURE GROUP</u>	<u>UNEXPOSED CONTROLS</u>
<u>Pituitary</u>			
Multilocular cysts	4	6	7
<u>Thyroid</u>			
C-cell hyperplasia	5	6	8
Lymphocytic infiltration	0	1	0
<u>Thymus</u>			
Ultimobronchial cyst	4	2	3
Atrophy	0	0	1
<u>Hepatocyte</u>			
Vacuolization	6	4	6
Pigmentation	5	7	7
Clear cell change	1	0	0
<u>Heart</u>			
Endocardiosis	5	5	3
<u>Lung</u>			
Eosinophilic granuloma	1	0	0
Granulomatous inflammation	0	0	1
Fibrosis, multifocal	2	3	3
Squamous metaplasia	1	0	0
Acute inflammation	0	0	1
<u>Splenic Capsule</u>			
Nodular hyperplasia	2	3	2
Hemangioma	0	1	0
<u>Kidney</u>			
<u>Collecting Tubules</u>			
Mineralization	7	5	3
Casts	1	2	0
<u>Convoluted Tubules</u>			
Pigmentation	3	4	3
<u>Gall Bladder</u>			
Cystic hyperplasia	2	4	1
<u>Pancreas</u>			
Hypertrophy	1	3	0
<u>Nasal Mucosa</u>			
Chronic inflammation	0	3	0
<u>Alveolus</u>			
Acute inflammation	0	0	1
<u>Anus</u>			
Adenocarcinoma	0	1	0
<u>Uterus^a</u>			
Distension	0	2	0
Subserosa cyst	0	1	2
Endometrial cyst	0	1	0
<u>Testis^a</u>			
Senile atrophy	1	2	2
<u>Prostate^a</u>			
Cyst	0	0	1

^aN = 4

There will be no attempt here to attach significance to the findings of tumors in one 0.25 ppm exposed dog and the hepatocytic clear cell change seen in the liver of one 1.0 ppm hydrazine exposed dog. These findings, along with the remainder of the non-neoplastic lesions seen in the incidence table, are interpreted as incidental findings not unusual in dogs of this age.

Lesions occurring in hamsters as a result of exposure to hydrazine were discussed in the last annual report. Surviving rats and mice were sacrificed 18 months postexposure and examined for neoplastic and nonneoplastic changes. The only tumors which showed increased incidences in male and female rats were those of the nasal cavity and, possibly, thyroid adenocarcinomas in male rats as shown in Tables 4 and 5. Certainly, both benign and malignant tumors of the nasal turbinates increased as a result of exposure, and benign tumors showed dose dependence in both sexes.

Varying degrees of acute inflammation were observed in the nasal cavity, larynx and /or trachea in some rats from the control and all treated groups. The incidence and severity of the inflammatory changes were greatest in male and female rats from the group receiving 5.0 ppm, and in some of these affected animals, they were associated with focal hyperplasia and/or squamous metaplasia of the epithelium of the nasal cavity, larynx, and trachea. These histopathologic changes were observed in rats dying during the study as well as at the 2 1/2 year terminal sacrifice.

The more severe grades of chronic respiratory disease were observed in lungs of some rats exposed to 5.0 ppm hydrazine and to a lesser degree in males exposed at 0.05 ppm. None of the males or the females exposed to 0.25 and 1.0 ppm showed epithelial hyperplasia. The morphological changes included peribronchial/peribronchiolar lymphoid hyperplasia, pneumonia, bronchopneumonia, and bronchiectatic abscesses.

The incidence of focal liver cell hyperplasia tended to be greater in treated as compared to control female rats at exposure levels of 1.0 ppm and 5.0 ppm. This effect was seen in female rats dying during the study, but not in female rats killed at the 2 1/2 year terminal sacrifice. There was no difference in the incidence of liver cell hyperplasia in treated as compared to control male rats.

There was no evidence that treatment with hydrazine increased the incidence of hepatic neoplasia. It was considered, therefore, that the slightly greater incidence of liver cell hyperplasia in treated as compared to control female rats arose fortuitously and that it was not related to treatment.

Acute endometritis was noted more frequently in female rats from the group receiving 5.0 ppm than in the controls or in rats

from the groups receiving 0.05 ppm, 0.25 ppm, or 1.0 ppm. Acute salpingitis was present only in rats from the highest dosage group with the exception of one female from the 1.0 ppm dosage level killed at termination.

TABLE 4. SELECTED TUMORS FOUND IN FEMALE FISCHER 344 RATS AFTER INHALATION EXPOSURE TO HYDRAZINE

TUMOR TYPE	UNEXPOSED CONTROLS (N=147)	EXPOSED 0.05 PPM (N=99)	EXPOSED 0.25 PPM (N=100)	EXPOSED 1.0 PPM (N=97)	EXPOSED 5.0 PPM (N=98)
Nasal Cavity:					
Epithelial (Benign)	0 (0)	1 (1)	0 (0)	4 (4) ^b	31 (32) ^a
Epithelial (Malignant)	0 (0)	0 (0)	0 (0)	0 (0)	5 (5) ^a
Pituitary:					
Adenoma	59 (40)	28 (28)	35 (35)	33 (34)	40 (41)
Adenocarcinoma	9 (6)	6 (6)	2 (2)	6 (6)	6 (6)
Thyroid:					
Adenoma	9 (6)	2 (2)	4 (4)	7 (7)	7 (7)
Carcinoma	17 (12)	1 (1)	8 (8)	15 (15)	5 (5)
Adrenals:					
Pheochromocytoma	10 (7)	3 (3)	6 (6)	9 (9)	12 (12)
Uterus:					
Adenoma	1 (0)	0 (0)	0 (0)	2 (2)	3 (3)
Adenocarcinoma	10 (7)	4 (4)	5 (5)	7 (7)	6 (6)
Endometrial stromal sarcoma	0 (0)	2 (2)	1 (1)	1 (1)	3 (3)
Lymphoreticular Tissue:					
Leukemias	41 (28) ^a	18 (18)	21 (21)	13 (13)	13 (13)
Sarcomas	4 (3)	4 (4)	4 (4)	2 (2)	6 (6)
Mammary gland:					
Adenoma	4 (3)	4 (4)	6 (6)	8 (8)	8 (8)
Fibroadenoma	28 (19)	20 (20)	11 (11)	18 (19)	19 (19)
Adenocarcinoma	2 (1)	1 (1)	2 (2)	2 (2)	3 (3)
Liver:					
Liver cell tumor	3 (2)	0 (0)	0 (0)	6 (6)	3 (3)
Lung:					
Bronchial adenoma	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)

^a Significant at the 0.01 level, control vs. test.

^b Significant at the 0.05 level, control vs. test.

() = % incidence.

TABLE 5. SELECTED TUMORS FOUND IN MALE FISCHER 344 RATS AFTER INHALATION EXPOSURE TO HYDRAZINE

TUMOR TYPE	UNEXPOSED CONTROLS (N=149)	EXPOSED 0.05 PPM (N=99)	EXPOSED 0.25 PPM (N=99)	EXPOSED 1.0 PPM (N=98)	EXPOSED 5.0 PPM (N=99)
Nasal Cavity:					
Epithelial (Benign)	0 (0)	2 (2)	2 (2)	10 (10) ^a	66 (67) ^a
Epithelial (Malignant)	0 (0)	1 (1)	0 (0)	1 (1)	6 (6) ^a
Pituitary:					
Adenoma	62 (42)	31 (31)	29 (29)	27 (28)	26 (26)
Adenocarcinoma	4 (3)	0 (0)	5 (5)	4 (4)	5 (5)
Thyroid:					
Adenoma	15 (10)	5 (5)	7 (7)	9 (9)	2 (2)
Adenomacarcinoma	7 (5)	6 (6)	5 (5)	9 (9)	13 (13) ^b
Adrenals:					
Pheochromocytoma	16 (11)	14 (14)	13 (13)	18 (18)	11 (11)
Testes:					
Interstitial cell tumor	104 (70)	80 (81)	73 (74)	83 (85)	74 (75)
Prostate:					
Squamous carcinoma	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Liver:					
Liver cell tumors	9 (6)	11 (11)	8 (8)	6 (6)	4 (4)
Lung:					
Bronchial adenoma	0 (0)	0 (0)	0 (0)	0 (0)	3 (3)
Lymphoreticular Tissue:					
Leukemias	36 (24)	20 (20)	28 (28)	22 (22)	10 (10) ^b
Sarcomas	8 (5)	9 (9)	3 (3)	6 (6)	3 (3)

^a Significant at the 0.01 level, control vs. test.

^b Significant at the 0.05 level, control vs. test.

() = % incidence.

Many microscopic variations from normal were seen in the aging mice of both control and hydrazine exposed groups. The only lesion of significance, an increased incidence of pulmonary adenomas in the 1.0 ppm hydrazine exposed mice, is shown in Table 8. This small increase in tumor incidence over unexposed control mice is similar to that previously reported in Swiss mice (MacEwen et al., 1974). An increased incidence of ovarian tubular adenomas was also noted in the group of mice exposed to 1.0 ppm hydrazine. This increase was not significant at the 0.05 confidence level, and its biological significance is uncertain.

TABLE 6. PATHOLOGIC CHANGES SEEN IN FEMALE FISCHER 344 RATS AFTER INHALATION EXPOSURE TO HYDRAZINE

TYPE OF LESION	UNEXPOSED CONTROLS	EXPOSED 0.05 PPM	EXPOSED 0.25 PPM	EXPOSED 1.0 PPM	EXPOSED 5.0 PPM
Nasal: Squamous meta- plasia	28/145(19)	18/97(19)	23/98(23)	24/94(26)	28/95(29) ^b
Epithelial hyperplasia	3/145(2)	2/97(2)	4/98(4)	5/94(5)	9/95(9) ^b
Larynx: Squamous meta- plasia	6/138(4)	2/91(2)	2/91(2)	4/91(4)	14/91(15) ^a
Inflammation	22/38(16)	11/91(12)	4/19(4)	10/91(11)	48/91(53) ^a
Trachea: Squamous meta- plasia	0/147(0)	0/96(0)	0/97(0)	0/95(0) ^b	6/98(6) ^a
Inflammation	0/147(0)	3/96(3)	1/97(1)	4/95(4)	29/98(30) ^a
Lung: Epithelial hyperplasia	0/147(0)	0/97(0)	0/100(0)	1/97(1)	3/98(3)
Adenomatous	7/147(5)	3/97(3)	3/100(3)	4/97(4)	3/98(3)
Heart: Myocardial degeneration	125/147(85)	82/97(85)	91/100(91)	83/97(86)	89/98(91)
Myocardial fibrosis	49/147(33)	24/97(25)	24/100(24)	26/97(27)	23/98(23)
Thymus: Regression	85/147(58)	55/97(57)	59/100(59)	46/97(47)	50/98(51)
Lymph Nodes: Hyperplasia	3/147(2)	2/97(2)	4/100(4)	3/97(3)	11/98(11) ^a
Liver: Hepatocyte degeneration	18/147(12)	15/97(15)	14/100(14)	13/97(13)	15/98(15)
Hepatic hyperplasia	57/147(39)	42/97(43)	36/100(36)	58/97(60) ^a	64/98(65) ^a
Kidney: Progressive glomerulo- nephrosis	82/147(56)	34/97(35)	52/100(52)	54/97(56)	79/98(81) ^b
Uterus: Polyps	26/147(18)	23/97(24)	21/100(21)	19/97(20)	19/98(19)
Cystic endo- metrial hyper- plasia	2/147(1)	1/97(1)	4/100(4)	1/97(1)	7/98(7) ^b
Endometritis	8/147(5)	5/97(5)	0/100(0)	6/97(6)	21/98(21) ^a
Squamous meta- plasia	3/147(2)	1/97(1)	1/100(1)	0/97(0)	2/98(2)
Ovary: Atrophy	15/149(10)	13/97(13)	3/100(3)	15/97(15)	22/98(22) ^b
Oviduct: Salpingitis	0/147(0)	0/97(0)	0/100(0)	1/97(1)	20/98(20) ^a

^a Significant at 0.01 level, control vs. test

^b Significant at 0.05 level, control vs. test

() = % incidence.

TABLE 7. PATHOLOGIC CHANGES SEEN IN MALE FISCHER 344 RATS AFTER INHALATION EXPOSURE TO HYDRAZINE

TYPE OF LESION	UNEXPOSED CONTROLS	EXPOSED 0.05 PPM	EXPOSED 0.25 PPM	EXPOSED 1.0 PPM	EXPOSED 5.0 PPM
Nasal:					
Squamous meta- plasia	24/146(16)	19/96(20)	24/94(26)	25/97(26)	47/99(47) ^a
Epithelial hyperplasia	4/146(3)	9/96(9) ^b	3/94(3)	4/94(4)	21/99(21) ^a
Larynx:					
Squamous meta- plasia	2/141(1)	2/95(2)	2/91(2)	3/91(3)	18/92(20) ^a
Inflammation	14/141(9)	42/95(44) ^a	7/91(8)	14/91(15)	72/92(78) ^a
Trachea:					
Squamous meta- plasia	0/145(0)	0/97(0)	0/98(0)	0/95(0)	10/97(10) ^a
Inflammation	5/145(3)	17/97(18) ^a	2/98(2)	2/95(2)	52/97(54) ^a
Lung:					
Epithelial hyperplasia	0/149(0)	6/99(6) ^a	0/99(0)	0/98(0)	6/99(6) ^a
Adenomatosis	6/149(4)	9/99(9)	7/99(7)	9/98(9)	4/99(4)
Heart:					
Myocardial degeneration	98/149(66)	71/99(72)	73/99(74)	76/98(78) ^b	82/99(83) ^a
Myocardial fibrosis	104/149(70)	67/99(68)	68/99(69)	73/98(74)	52/99(53)
Thymus:					
Regression	67/149(45)	43/99(43)	57/99(58) ^b	48/98(49)	44/99(44)
Lymph Nodes:					
Hyperplasia	4/149(3)	5/99(5)	3/99(3)	5/98(5)	5/99(5)
Liver:					
Hepatocyte degeneration	18/149(12)	20/99(20)	16/99(20)	12/98(12)	7/99(7)
Hepatic hyperplasia	58/149(39)	39/99(39)	40/99(40)	41/98(42)	42/99(42)
Kidney:					
Progressive glomerulo- nephrosis	137/149(92)	90/99(90)	93/99(94)	90/98(92)	90/99(91)
Testes:					
Atrophy	119/149(80)	85/99(86)	77/99(78)	85/98(87)	84/99(85)
Interstitial hyperplasia	29/149(19)	12/99(12)	18/99(18)	11/98(11)	13/99(13) ^b
Prostate:					
Hyperplasia	8/149(5)	1/99(1)	11/99(11)	9/98(9)	13/99(13)

^a Significant at 0.01 level, control vs. test

^b Significant at 0.05 level, control vs. test

() = % incidence.

TABLE 8. NEOPLASTIC PATHOLOGY IN CONTROL AND HYDRAZINE EXPOSED FEMALE C57B1/6 MICE

TUMOR TYPE	Set No. 1			Set No. 2	
	Unexposed Controls (N=385)	Exposed 0.05 ppm (N=364)	Exposed 0.25 ppm (N=382)	Unexposed Controls (N=378)	Exposed 1.0 ppm (N=379)
<u>Pituitary</u>					
Adenoma	152 (39)	94 (26)	101 (26)	109 (29)	64 (17)
Carcinoma	7 (2)	10 (3)	3 (1)	8 (2)	2 (1)
<u>Thyroid</u>					
Adenoma	17 (4)	25 (7)	19 (5)	34 (9)	22 (6)
Carcinoma	2 (1)	1 (0)	1 (0)	2 (1)	1 (0)
<u>Uterus</u>					
Adenocarcinoma	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)
<u>Lymphoreticular Tissue</u>					
Leukemias	4 (1)	5 (2)	11 (3)	5 (1)	0 (0)
Sarcomas	145 (38)	154 (42)	150 (39)	154 (41)	139 (37)
<u>Mammary Gland</u>					
All tumors	1 (0)	1 (0)	0 (0)	1 (0)	0 (0)
<u>Liver</u>					
Liver cell tumor	4 (1)	9 (2)	6 (2)	6 (2)	11 (3)
<u>Lung</u>					
Adenoma	8 (2)	3 (1)	5 (1)	4 (1)	12 (3)*
Adenocarcinoma	2 (1)	1 (0)	2 (1)	3 (1)	3 (1)
<u>Ovary</u>					
Tubular adenoma	12/369(3)	10/340(3)	11/365(3)	13/365(4)	23/361(6)

* Significant at 0.05 level, control vs. test.
 () = % incidence.

The occurrence of nonneoplastic lesions in the C57B1/6 mice used in this study was similar in all groups with no apparent treatment effects.

Analysis of the oncogenic changes and other toxic effects of exposure to hydrazine indicates that the nononcogenic sequelae were more severe in producing debilitation and lethal effects. The oncogenic changes were observable only at the microscopic level producing little or no impairment of respiratory function and had no effect on life expectancy.

We conclude from this study that hydrazine vapor is a relatively weak tumorigen in rodents after inhalation concentrations of 1.0 and 5.0 ppm for one year. The incidence of benign and malignant tumors was highest in nasal turbinates of rats. This rat tissue has demonstrated extreme sensitivity to the action of respiratory carcinogens (HMPA and formaldehyde) and may not be directly extrapolatable to exposure of humans who are not obligate nose-breathers. Nevertheless, the toxic and oncogenic effects seen in this study indicate that the current OSHA Threshold Limit Value of 1.0 ppm for hydrazine is unsatisfactory and is

also too near concentrations which caused death in chronically exposed animals in previous studies. More realistically, the ACGIH recommended TLV of 0.1 ppm would be expected to provide adequate protection.

A STUDY OF THE ONCOGENIC POTENTIAL OF PURIFIED UDMH IN MICE

UDMH (1, 1-dimethylhydrazine) is a missile fuel used by the military which has been reported to be carcinogenic in animals (Roe, 1967; Toth, 1972 and 1973) when administered orally or by gavage.

The indication that UDMH is carcinogenic has led to studies involving the inhalation of UDMH vapors. MacEwen and Vernot (1976) described a study involving the exposure of four animal species to UDMH vapors. Dogs exposed to 5.0 ppm UDMH for a period of six months on an intermittent (5 day/week, 6 hours/day) schedule exhibited hepatotoxic symptoms characterized by increased SGPT levels and increased BSP retention time. Histologic examination of the tissues of rodents exposed to UDMH indicated that mice were the most susceptible species with significant increases in circulatory and liver tumors in exposed animals.

The UDMH used for the study described by MacEwen and Vernot (1976) contained 0.12% dimethylnitrosamine (DMNA) which was used as a starting material in the manufacturing of UDMH. Haun (1976) suggested that the hepatotoxic effects described by MacEwen and Vernot (1976) were due to the presence of the DMNA in the sample of UDMH used for the inhalation exposure. He conducted inhalation studies in dogs using either purified UDMH or UDMH spiked to contain 0.12% DMNA. He concluded from the results that the hepatotoxic effects observed by MacEwen and Vernot (1976) were related to the DMNA present in the UDMH.

The incidence of tumors in the mice exposed to UDMH by MacEwen and Vernot cannot be conclusively associated with UDMH since there was simultaneous exposure to DMNA. DMNA is a potent liver toxin which suggests that it, rather than UDMH, may have been the agent responsible for the tumors.

In an attempt to resolve the uncertainty in interpretation of the previous chronic inhalation results, the present study was designed to use UDMH of as high a purity as possible in a year-long exposure to the highest concentration used previously, 5.0 ppm. The UDMH was redistilled from propellant grade UDMH and contained a DMNA concentration of less than 5 $\mu\text{g}/\text{ml}$. Since mice had been most susceptible to the oncogenic effects of exposure, it was determined that they would be the only species tested.

Two hundred female mice (C57B1/6) were exposed for one year to 5.0 ppm purified UDMH in 2 Rochester inhalation exposure

chambers 2 m³ in volume. The exposures were conducted following an industrial work week type schedule of 5 days/week, 6 hours/day. An equal number of mice serving as controls was maintained in Longley chambers. At the conclusion of the exposure, all mice were removed from the chambers and housed in Bio-Clean® laminar air flow facilities for one year of postexposure observation. At the end of the observation period, all remaining mice will be sacrificed. The tissues from these mice as well as the tissues from mice dying during the observation period will be taken for histologic evaluation of tumorigenesis.

A description of the UDMH vapor generation and analysis system is described in the previous annual report (MacEwen and Vernot, 1979).

The exposure phase of the study was completed in June of 1979. The effect of UDMH exposure on mouse body weight is shown in Figure 1. Differences in mean body weights between 5.0 ppm UDMH exposed mice and unexposed control mice were evident at 7 months of exposure. These differences have continued through the 10th month of postexposure observation at which time there is a six gram difference between the test and control group body weight means.

The cumulative mortality of the mice is shown in Figure 2. Exposure to 5.0 ppm UDMH has had negligible effect on mouse mortality.

Two extra mice had been included in the exposed and control groups for electron microscopic examination midway through exposure. These were removed after six months exposure according to plan. Lungs were collected from each animal, and the bronchi, respiratory bronchioles, and alveoli were examined using the scanning electron microscope. Livers were also collected and examined using transmission electron microscopy. All specimens of lung or liver tissue were morphologically normal. There were no differences observed between 5.0 ppm UDMH exposed and unexposed control mice.

Histopathologic examination of the tissue of animals dying during the study has not been completed. All remaining animals will be sacrificed in June 1980 for tissue collection and examination.

1,2-DIMETHYLHYDRAZINE DIHYDROCHLORIDE AS A POSITIVE TUMORIGENIC CONTROL FOR THE EXPERIMENTAL ANIMAL SPECIES USED IN CURRENT ONCOGENIC STUDIES

Oncogenic studies performed in this laboratory on the hydrazine compounds utilized specific strains and sexes of the various rodent species. These were female C57B1/6 mice, male and female

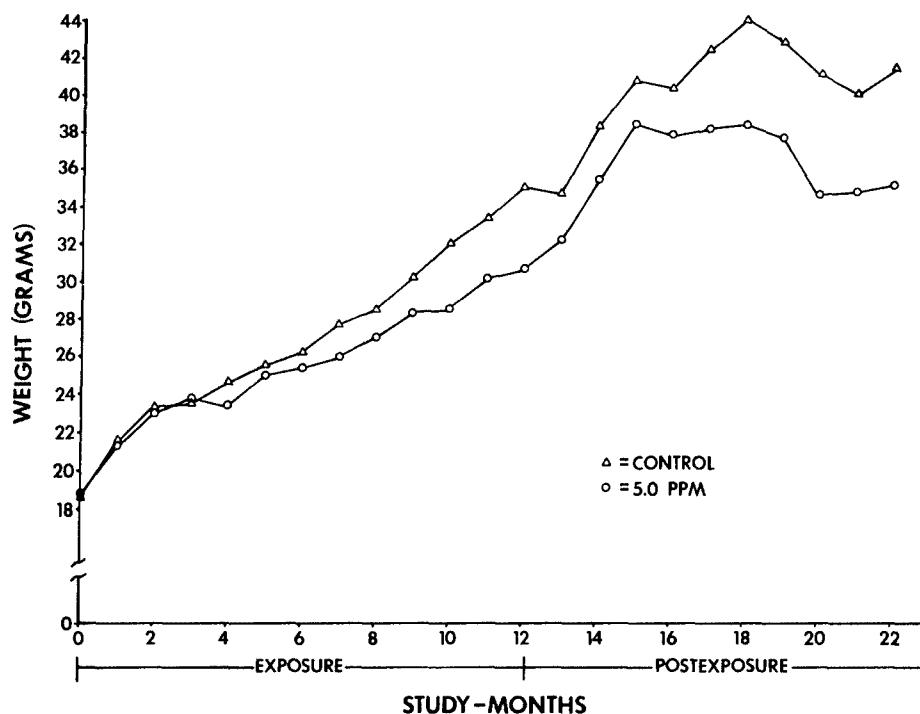


Figure 1. Effect of inhaled UDMH on mouse body weight.

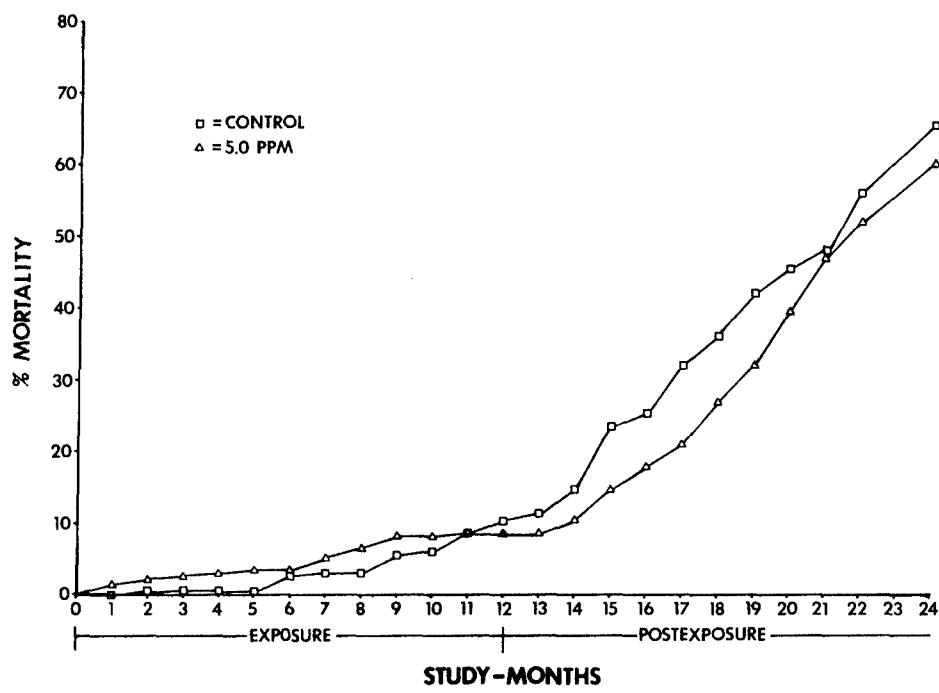


Figure 2. Mortality of mice exposed to UDMH for 1 year.

Fischer 344 rats and male Golden Syrian hamsters. Since the response of these animals to a known carcinogen had not been measured in our laboratory, this study was designed to measure that response and to serve as a positive control for a concurrent MMH study. 1,2-dimethylhydrazine which produces lung, liver, blood vessel and digestive system tumors in experimental animals, was selected for the purpose and delivered subcutaneously as the dihydrochloride (SDMH · 2 HC1) buffered to pH 6.8.

The 1978 annual report (MacEwen and Vernot, 1978) defines the materials and methods used in this study as well as the details concerning the subcutaneous rangefinding LD₅₀ determinations done on each species of animal. The rangefinding LD₅₀ values and 95% confidence limits are shown below:

Mice:	38.2 (34.6 - 42.4) mg/kg
Hamsters:	50.0 (44.4 - 56.0) mg/kg
Rats, Male:	121.8 (70.4 - 158.4) mg/kg
Rats, Female:	125.8 (93.1 - 169.8) mg/kg

The toxic response of the hamsters was very similar to that in the mouse. The rats, however, were more resistant with no differences noted between the sexes.

Initially, 20 mg/kg SDMH·2 HC1 was selected as a weekly dose which should have produced tumors without causing significant early mortality. The hamsters were unable to tolerate a weekly dose of 20 mg/kg, and all died within fifteen weeks. Therefore, it was necessary to lower the dose for hamsters to 10 and 5 mg/kg. Body weight effects were noticed soon after beginning the study in the hamsters and male rats when compared to their respective controls. The 10 mg/kg dosed group showed a sharp decrease in mean body weight following 16 weekly injections and the group given 5 mg/kg weekly did not gain weight at the same rate as untreated control hamsters. The mean body weights of the female rat group did not show any difference from controls until after 36 weekly injections.

Mortality in the various species exposed to SDMH was discussed in the last THRU annual report and time to attain 90% mortality is shown in Table 9.

The 1979 annual report (MacEwen and Vernot, 1979) details the histopathologic findings of the mice and both sexes of rats. SDMH dihydrochloride induced malignant neoplasms of the vascular endothelium and of intrahepatic bile ducts in C57B1/6 female mice. Benign neoplasms were induced in the colonic mucosa. A high number of malignant neoplasms was seen in the test group of rats while none was seen in the respective control groups. Many of the malignancies were found in the mucosal epithelium of the

TABLE 9. MORTALITY RESPONSE TO WEEKLY SUBCUTANEOUS INJECTIONS OF SDMH DIHYDROCHLORIDE

<u>Species & Sex</u>	<u>Original Number</u>	<u>Weekly Dose (mg/kg)</u>	<u>Weeks to First Death</u>	<u>Weeks to 90% Mortality</u>
Rats, Male	25	20	19	39
Rats, Female	25	20	25	51
Mice, Female	50	20	25	44
Hamsters, Male	50	10	19	33
Hamsters, Male	50	5	24	52 ^a

^a Study terminated at one year with 84% mortality.

gastrointestinal system. There were also 26 malignant neoplasms seen in the Zymbal's gland.

During this report period, results of the histopathologic examinations of hamster tissues became available. Several types of lesions were identified and are shown in Table 10. The predominant tumor was one seen in blood vessels (hemangiosarcoma) of lung and liver which were found only in the SDMH treated hamsters. The incidence of these blood vessel tumors was 84% in the 5 mg/kg SDMH treated group and 60% in the 10 mg/kg exposure groups and shown in Table 10. This inverse dose response is probably due to the early deaths of many hamsters in the high-dose group before they could develop tumors. A 12% incidence of colonic adenocarcinoma was seen in the 10 mg/kg SDMH exposure group but not in the lower dosed hamsters or their untreated controls.

TABLE 10. INCIDENCE OF HISTOLOGIC LESIONS IN GOLDEN SYRIAN HAMSTERS AFTER WEEKLY INJECTION WITH SDMH AND THEIR UNEXPOSED CONTROLS

	<u>Untreated Control</u>	<u>Treatment Group 5 mg/kg</u>	<u>Treatment Group 10 mg/kg</u>
<u>Lung:</u>			
Inflammation	0/49 (0%)	2/50 (4%)	9/48 (19%)
Hemangiosarcoma	0/49 (0%)	0/59 (0%)	2/48 (4.2%)
<u>Liver:</u>			
Heptocellular Carcinoma	0/49 (0%)	1/49 (2%)	6/50 (12%)
Hemangiosarcoma	0/49 (0%)	41/49 (84%)	30/50 (60%)
Intrahepatic Bile Duct Hyperplasia	1/49 (2%)	17/49 (35%)	16/50 (32%)
Hepatocyte Necrosis	0/49 (0%)	10/49 (20%)	32/50 (64%)
Hemorrhages and Cysts	0/49 (0%)	21/49 (43%)	25/50 (50%)
Nodular Regeneration	0/49 (0%)	0/49 (0%)	2/50 (4%)
<u>Colon:</u>			
Adenocarcinoma	0/47 (0%)	0/40 (0%)	5/41 (12%)
<u>Kidney:</u>			
Mineralization	4/49 (8%)	12/49 (25%)	9/50 (18%)

Male and female Fischer 344 rats, female C57B1/6 mice and male Golden Syrian hamsters have all been shown in this study to develop malignant tumors when exposed to SDMH, a known carcinogen, indicating that the rodents used in our oncogenic studies of UDMH, MMH, and hydrazine are not tumor resistant and are suitable species for this purpose.

THE EVALUATION OF THE ONCOGENIC POTENTIAL OF
INHALED HYDRAZINE IN RATS AND HAMSTERS
AFTER A SHORT SERIES OF WEEKLY ONE-HOUR EXPOSURES

Hydrazine is used in operational aircraft as fuel in standby power systems. Maintenance of the systems may result in repeated accidental human exposure to high concentrations for brief periods. The specific concern and purpose of this study is to assess the oncogenic risk of this type of exposure to maintenance personnel. The design and conduct of this study will simulate severe human exposure utilizing total lifetime doses of hydrazine that caused pulmonary tumors and nasal polyps in rodents in previous inhalation studies.

Hydrazine was shown to be a weak oncogen for rats, mice and hamsters exposed to 5.0 ppm hydrazine 6 hours/day, 5 days/week for a one-year period (MacEwen et al., 1979). The calculated CT or dose equivalent value (concentration x time) for these exposures was 7500 ppm-hours. Actual human exposure would more likely be single or a few repeated brief exposures to high concentrations of hydrazine. In order to closely simulate possible human exposure, this study will utilize exposure periods of one hour to maximum nonlethal concentrations of hydrazine. Exposure will be limited to one per week to permit recovery from the acute effects. A sufficient number of one-hour exposures to the maximum nonlethal concentrations will be utilized to reach a CT of 7500 ppm-hours. Since the 7500 ppm-hour CT of hydrazine has already been demonstrated to produce nasal tumors in rats and hamsters, these species will be used.

This study is being conducted in three phases. The preliminary phase is the determination of the one-hour LC₅₀ values and maximum nonlethal concentration of hydrazine to male and female rats. This work has already been conducted on hamsters. The one-hour LC₅₀ of hydrazine to male hamsters was found to be 2585 ppm (MacEwen and Vernot, 1975).

The maximum nonlethal concentration found in Phase I will be used to calculate the number of one-hour exposures required to equal a CT of 7500 ppm-hours. Phase II will consist of the exposure of small numbers of rats and hamsters to this concentration for the required number of exposure periods. The primary purpose of Phase II is to confirm that the selected concentration of hydrazine is correct prior to committing large blocks of animals and time. Phase III involves the exposures of large numbers of rats and hamsters to the selected concentration for

the required number of exposures. In an attempt to establish a no-effect level, separate groups of rodents will be exposed to a CT of 750 ppm-hours.

After the series of Phase III weekly one-hour exposures is completed, the rodents will be maintained for 2 years to evaluate the oncogenic potential of hydrazine.

All animals, exposed and controls, will be weighed prior to exposure. Weighing will be done weekly in the case of the Phase II and Phase III animals. The Phase I animals will be weighed at 3, 7, 10, and 14 days postexposure. During the long-term post-exposure holding of Phase III animals, weighings will be monthly.

Necropsy will be conducted on all animals that die or are sacrificed in Phases II and III. The following tissues will be taken for histopathologic examination from Phase III animals: nasal cavity, larynx, trachea, lungs, bronchi, and thyroid.

EVALUATION OF THE TOXIC EFFECT OF A 90-DAY CONTINUOUS INHALATION EXPOSURE TO PETROLEUM JP-4 VAPOR

Starting in 1977, a series of inhalation toxicity studies has been performed on Navy and Air Force hydrocarbon fuels in which dogs, rats and mice are exposed continuously for 90 days. All dogs and one-third of the rodents are sacrificed immediately following exposure for anatomic and clinical pathologic examination. The rest of the rodents are held for 19 months at which time half of the remaining animals are killed and examined clinically and anatomically. The final sacrifice is made when mortality reaches 90% of the original group number. Exposures to decalin, JP-5, petroleum and shale derived, and petroleum DFM have already taken place, and exposure to shale DFM was being planned at the time this study was begun. This experiment was designed to include information from petroleum JP-4 in the subchronic 90-day continuous exposure data base.

JP-4 is a complex mixture of aliphatic and aromatic hydrocarbon compounds defined in terms of physical and chemical characteristics and including various additives, all of which meet the requirements of Military Specification MIL-J-5624E. Upper limits for some of the constituents are detailed in the military specifications listed below:

<u>Constituent</u>	<u>Max. Conc.</u>
Sulfur	0.4% (by wt.)
Mercaptan Sulfur	0.001% (by wt.)

Aromatics	25.0% (by vol.)
Olefins	5.0% (by vol.)
Various Butyl Phenol Antioxidants	24 mg/liter
Aliphatic Diamine Metal Deactivators	5.8 mg/liter

These constituents represent only a fraction of the total content of JP-4 jet fuel, the remainder consists of unspecified hydrocarbon compounds in the kerosene boiling range.

The concentrations selected, 1000 and 500 mg/m³, were chosen after analysis of the benzene concentration of the lot of JP-4 available. These concentrations of JP-4 insured that the level of benzene in the chambers would not exceed that equivalent to a 6-hour time-weighted average (TWA) of 10 ppm.

Each chamber contained 3 male and 3 female purebred beagle dogs, 150 female mice, 75 male and 75 female rats. Another group with the same number of animals was held at the Veterinary Sciences Division Building (Vivarium) to serve as unexposed controls. Animals were caged in conformance with ILAR standards for laboratory care. The experimental animals were randomized from the main group after quality control procedures and quarantine had been completed. Assignment of the animals from each species to each group was made by use of the THRU computer program RANDUM which utilizes the Fortran library subroutine RANF (X).

Upon termination of the 90-day exposure, all dogs and one third of the rodents (50 female mice, 25 male rats, 25 female rats) from each exposure group and controls were sacrificed for gross and histopathologic examination to detect any lesions caused by exposure to JP-4 vapors. One-half of the remaining rodents will be sacrificed at 19 months postexposure for gross and histopathologic examination. These animals should have attained a normal lifetime age without a large number of deaths occurring and should provide statistically satisfactory samples of animal tissues which will not be compromised by cannibalism or postmortem degeneration. The remaining rodents will be held until mortality of the species reaches 90% of the original 150 animals. At that time, all representatives of that species will be sacrificed for gross and histopathologic examination. All animals that died during exposure or were sacrificed at 90 days were necropsied and major organs taken for histopathologic examination. Animals dying during the postexposure period are necropsied in accordance with the NCI protocol, harvesting 33 tissues.

Pure-bred beagle dogs used in this study were provided by the Air Force. These dogs had been quarantined, and background clinical data were collected. Rodents used are listed below:

<u>Species</u>	<u>Strain</u>	<u>Source</u>
Rat	Fischer 344	Charles River Breeding Labs
Mouse	C57B1/6	Jackson Laboratory

All animals were fed ad libitum and the cage areas cleaned daily.

The contaminant introduction system for JP-4 was similar to the systems used for the previous fuel studies. Figure 3 illustrates the introduction system. The liquid material was pumped under low pressure from a 55-gallon supply drum and then passed through flowmeters to glass evaporator columns heated to an air temperature not greater than 120F. The air stream flowing through the evaporator carried the vapors into the main air supply for the chambers. Excess fuel which was not vaporized in the evaporator was drained into the receiving tank where it was collected for disposal.

Thermocouples were placed at the top and bottom of the glass evaporator to sense any hazardous increase in temperature and to activate both an alarm and a solenoid valve system which would cut off the fuel supply (Figure 4).

Continuous analysis of the chamber concentrations was done by pumping air samples from each dome into a total hydrocarbon analyzer. The sampling system for analysis is detailed in Figure 5. During previous testing of fuels by this laboratory, it was found that propane gave the same hydrocarbon detector sensitivity as the fuels. Therefore, known propane concentrations were used as the calibration standards. A daily span check of the hydrocarbon analyzer was made using a prepared tank of propane.

Output of the vapors by the generation system was a function of the fuel flow rate, air flow rate, and temperature. Under defined operating conditions, the output was stable. Therefore, hourly checks were made to assure that the predetermined settings were maintained.

Gas chromatograms of the vapors obtained from the headspace of each drum of JP-4 were made prior to start of the study. A GC fingerprint of the contaminant in the dome was also obtained when a new drum was put into service as a source of JP-4 vapors. A Royco® particle counter equipped with a 508 digital monitor was used to measure possible formation of vapor condensate aerosol.

All animals were observed hourly during the exposure and daily thereafter. Animals found in a moribund condition were sacrificed to reduce incidence of postmortem degeneration and cannibalism as much as possible.

Rats were individually weighed at biweekly intervals during exposure and are being weighed monthly during the postexposure

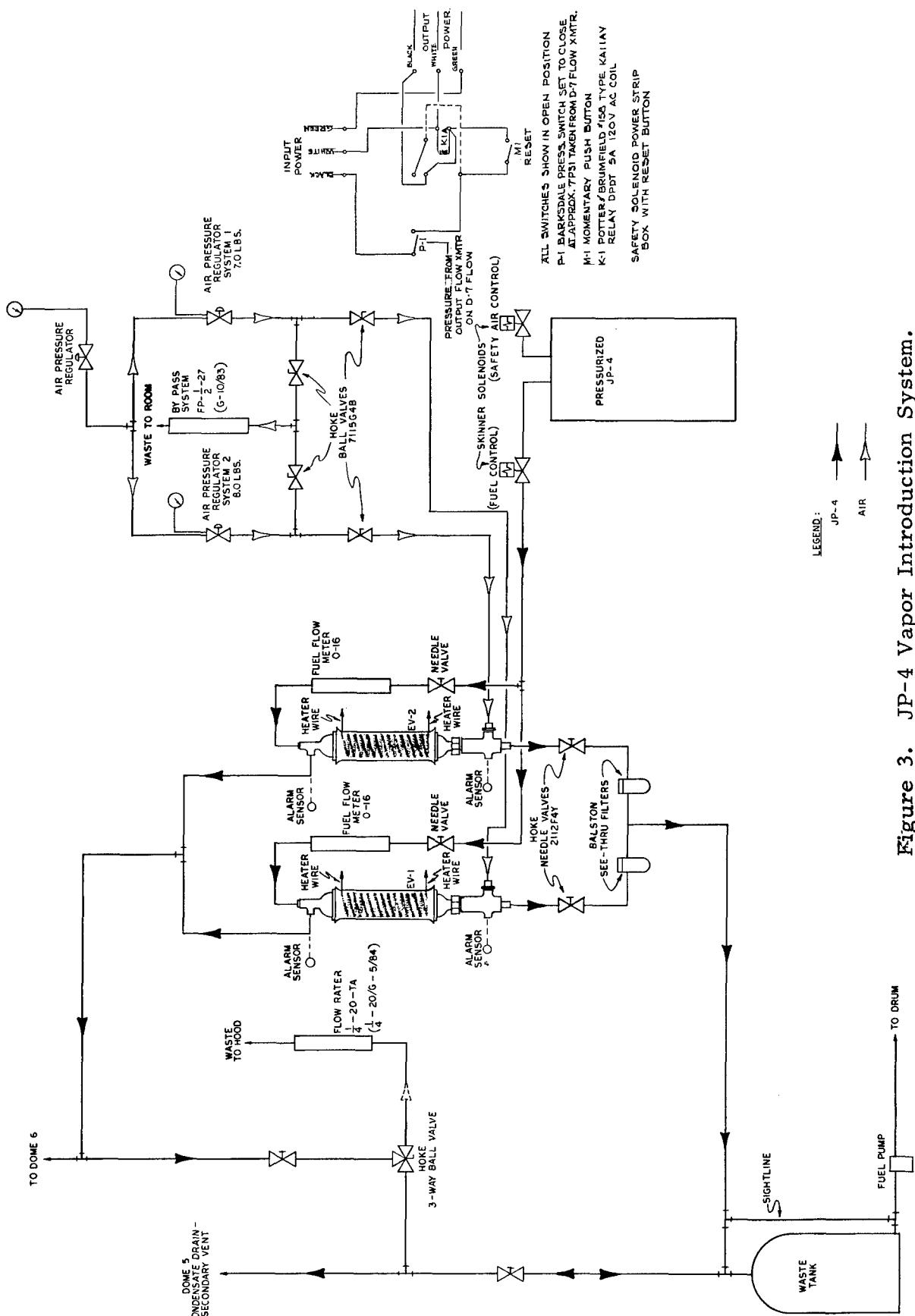


Figure 3. JP-4 Vapor Introduction System.

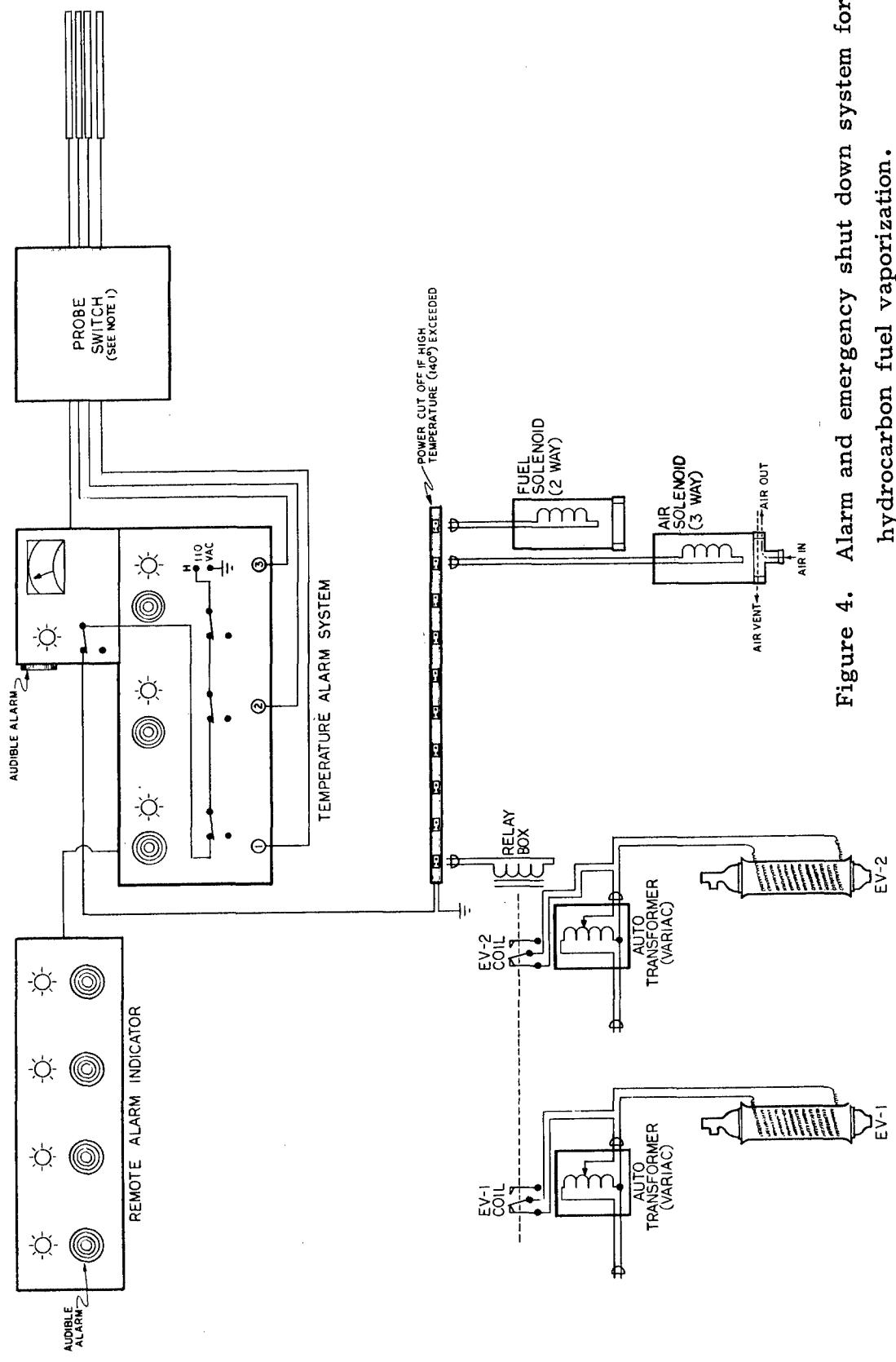


Figure 4. Alarm and emergency shut down system for hydrocarbon fuel vaporization.

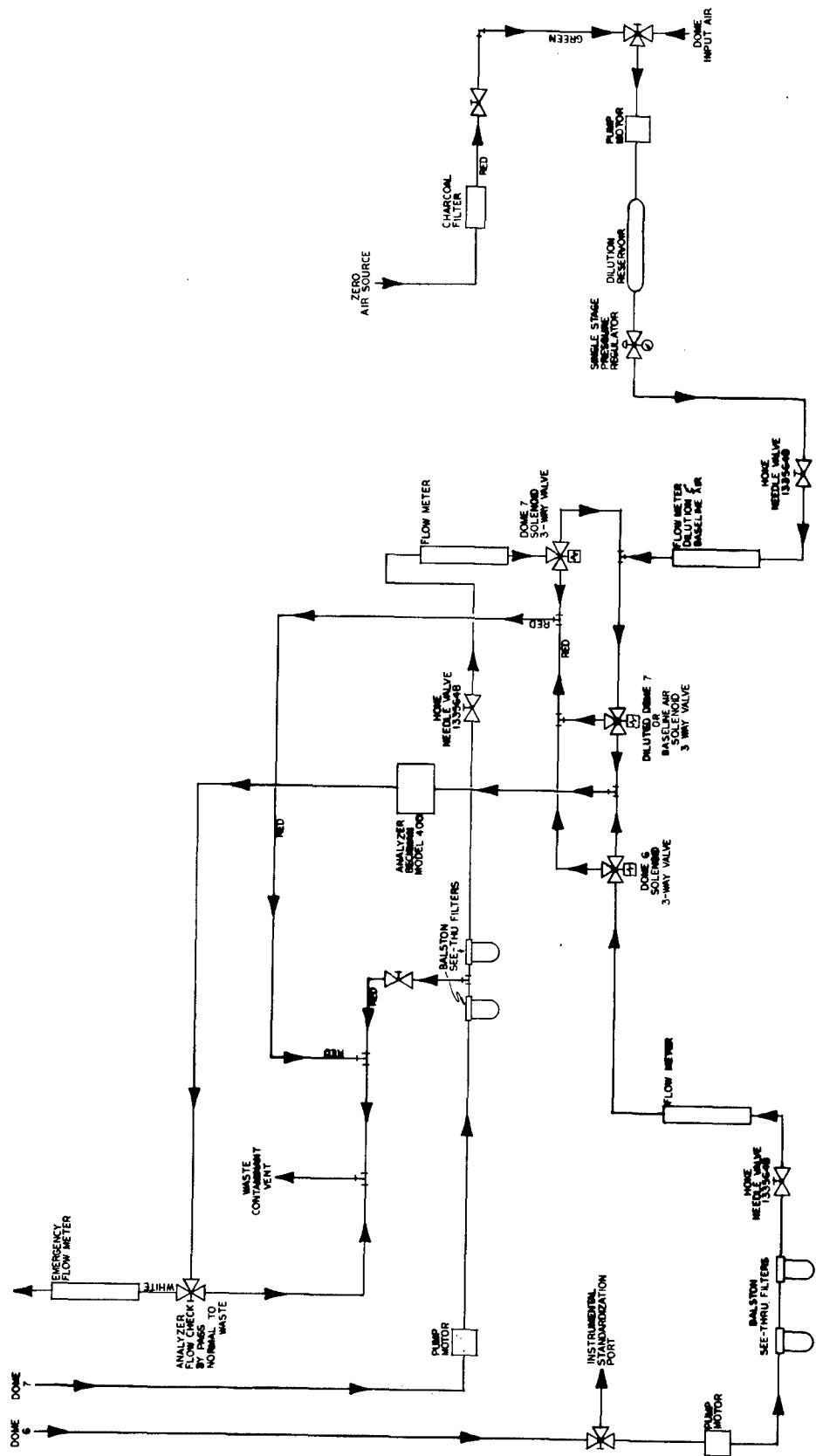


Figure 5. Inhalation chamber analysis system for hydrocarbon fuels.

period. Dogs were individually weighed at biweekly intervals during the exposure. Mice are weighed in groups with the group mean weights being followed on a monthly basis throughout the experimental period.

Blood samples were drawn from the dogs at biweekly intervals and clinical determinations made for the series of tests shown in Table 11. Additional blood samples were drawn monthly for red blood cell fragility tests.

TABLE 11. CLINICAL HEMATOLOGY AND CHEMISTRY TESTS PERFORMED ON DOGS AND RATS EXPOSED TO PETROLEUM JP-4 VAPOR

<u>Hematology</u>	<u>Chemistry</u>
Hematocrit	Sodium
Hemoglobin	Potassium
RBC	Calcium
WBC	Albumin/Globulin
Differential	Total Protein
Mean Corpuscular Volume (MCV)	Glucose
Mean Corpuscular Hemoglobin (MCH)	Alkaline Phosphatase
Mean Corpuscular Hemoglobin Concentration (MCHC)	SGPT
RBC Fragility	SGOT
	Bilirubin
	Creatinine
	BUN

Blood was taken via the portal vein from the rats at sacrifice for the tests shown in Table 11. Approximately 5 ml of whole blood is required to complete these tests. Once the blood was drawn, it was centrifuged within one hour and serum removed. If any of the samples were hemolyzed, they were discarded and a note of this made on the blood test report.

Liver, spleen, and kidney weights were obtained from the dogs and rats that were necropsied following exposure. Similar weights will be obtained from animals examined at the scheduled 19-month sacrifice.

Clinical chemistry determinations of the dog blood showed an increase in globulin (Figure 6) with a resultant increase in total protein (Figure 7) values. The increase in total protein was statistically significant at two and eight weeks in the 500 mg/m^3 group and at eight and 12 weeks in the 1000 mg/m^3 group. Although the values drop at the beginning of the study, the test values increase dramatically when compared to the control values after two weeks of exposure. Albumin values of all groups remained normal throughout the 12-week exposure period.

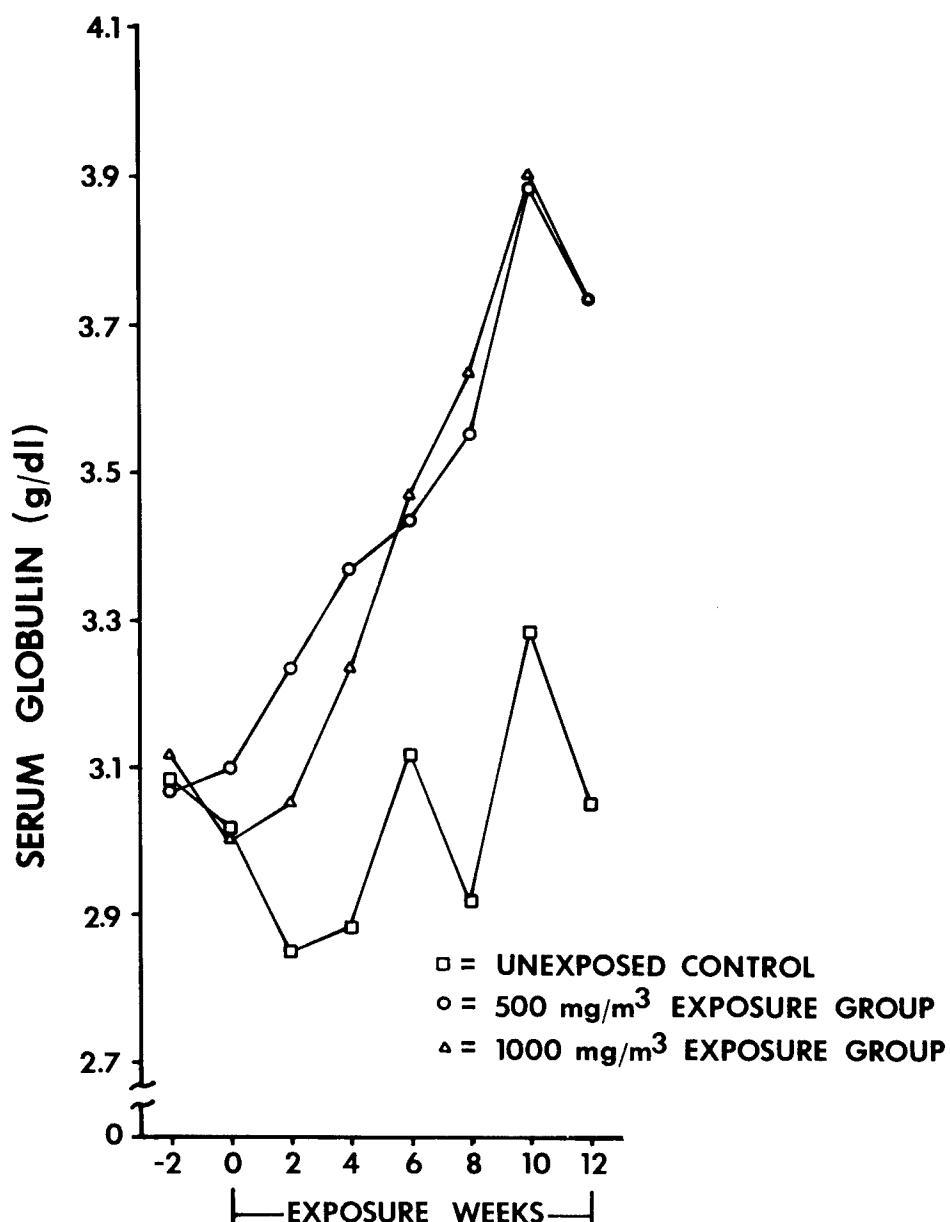


Figure 6. Effect of 90-day continuous inhalation exposure to petroleum JP-4 vapor on serum globulin content of beagle dog blood.

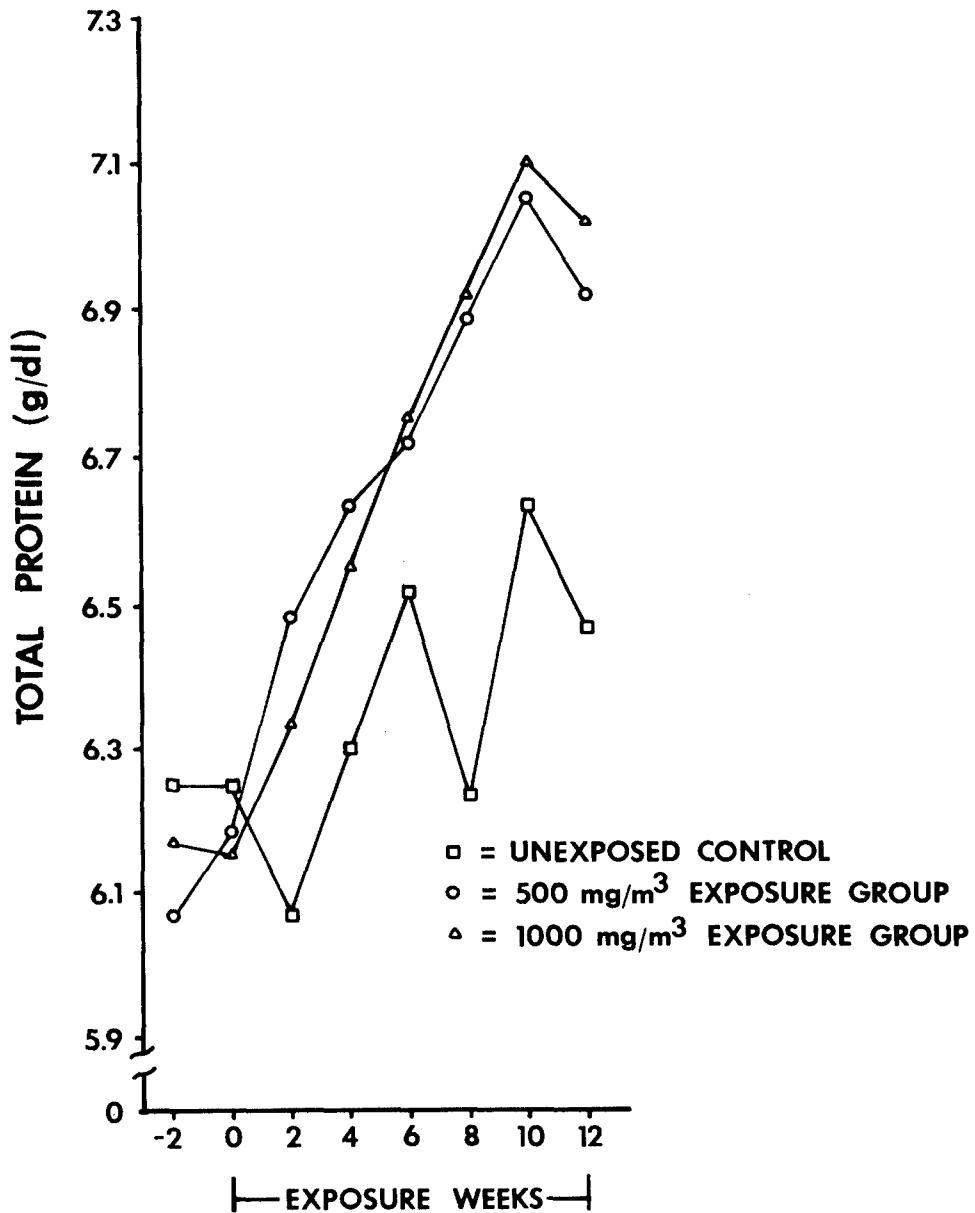


Figure 7. Effect of 90-day continuous inhalation exposure to petroleum JP-4 vapor on serum total protein content of beagle dog blood.

The blood urea nitrogen (BUN) values of the test dogs (Figure 8) showed an increase over the values of the control dogs which started at two weeks of exposure and continued through termination of the study. The exceptionally high value at six weeks appears to be a transient finding not associated with exposure. Although all three of these blood parameters were significantly different from controls, the values were still within normal biological limits for the species.

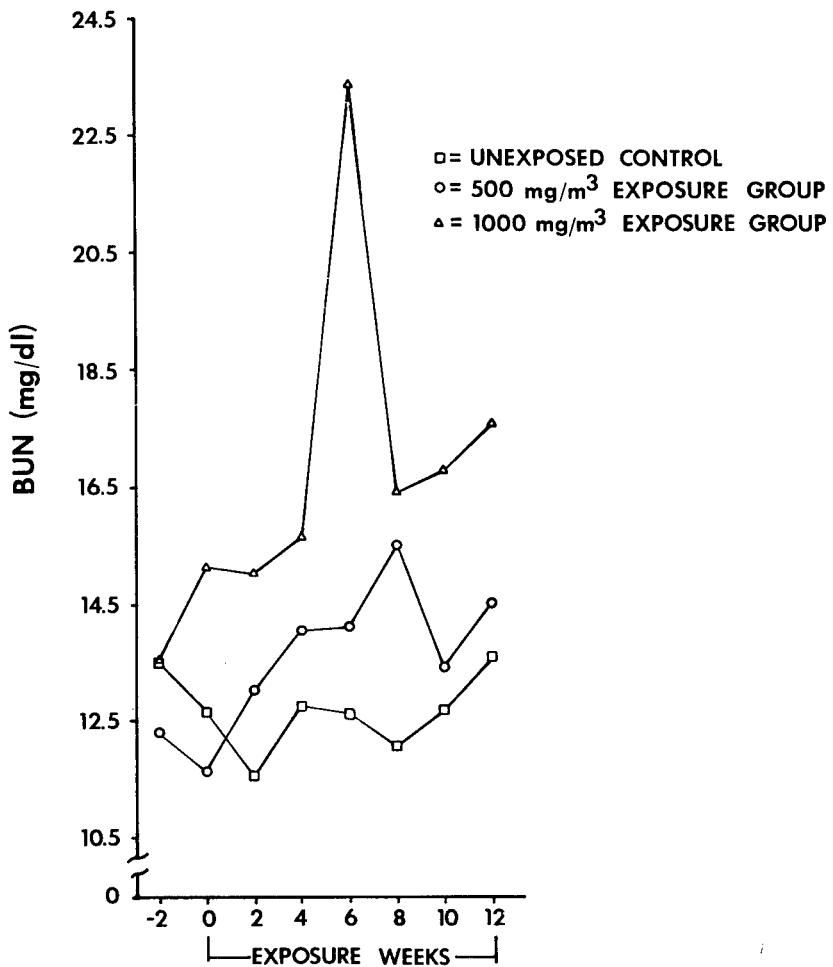


Figure 8. Effect of 90-day continuous inhalation exposure to petroleum JP-4 vapor on blood urea nitrogen (BUN) content in beagle dogs.

The results of the RBC osmotic fragility tests are shown in Table 12. The statistically significant difference shown at 4 weeks, at the highest saline concentrations, is caused primarily by abnormally low control values. There appears to be no significant effect on this parameter as a result of JP-4 exposure.

Dog organ weights, measured at sacrifice, are shown in Table 13. The average spleen weights and weight ratios of the test dogs differ significantly from the control dogs. The difference appears to result from the abnormally high weights of the spleens taken from the control dogs. The mean weight of the spleens from exposed dogs, as well as the spleen/body weight ratios, are not significantly different from those found in control dogs previously. This is shown by comparison with the average of spleen weights in control dogs at sacrifice (63 grams) after the last three 90-day continuous exposures.

TABLE 12. EFFECT OF 90-DAY CONTINUOUS EXPOSURE TO
PETROLEUM JP-4 VAPOR ON RED BLOOD CELL FRAGILITY^a
IN BEAGLE DOGS

Exposure Week	Control	500 mg/m ³	1000 mg/m ³	Saline Conc.
-2	2.8 + 1.9	2.8 + 2.0	4.1 + 4.7	0.50%
	5.2 + 3.6	2.9 + 1.4	3.8 + 3.2	
	2.1 + 0.9	4.7 + 1.8 ^b	7.0 + 10.4	
	3.9 + 2.8	5.1 + 6.0	5.2 + 7.9	
	4.8 + 4.2	5.5 + 3.2	6.1 + 8.2	
0	8.1 + 5.3	6.8 + 4.1	9.9 + 11.2	0.475%
	8.8 + 7.7	6.4 + 1.3	8.6 + 9.5	
	3.9 + 1.9	9.5 + 2.2 ^b	11.4 + 12.4	
	10.9 + 5.9	13.9 + 16.2	18.5 + 22.6	
	11.0 + 6.6	11.8 + 7.9	14.7 + 16.6	
4	22.6 + 13.0	22.5 + 9.6	23.9 + 21.8	0.45%
	20.1 + 14.5	18.3 + 5.4	22.5 + 19.5	
	13.3 + 5.8	17.5 + 3.1	24.2 + 20.3	
	15.6 + 6.9	25.9 + 22.4	28.5 + 30.4	
	21.3 + 11.1	27.4 + 17.6	26.9 + 22.3	
8	42.0 + 21.9	40.4 + 14.8	41.5 + 26.3	0.425%
	44.0 + 22.5	42.1 + 6.0	46.4 + 23.9	
	31.9 + 11.9	35.8 + 12.9	31.9 + 22.1	
	39.8 + 19.4	35.9 + 18.5	27.2 + 13.4	
	41.0 + 17.0	49.3 + 24.4	44.7 + 22.3	
12	63.7 + 21.3	70.9 + 9.9	63.9 + 25.8	0.40%
	61.0 + 19.9	65.5 + 4.7	64.6 + 25.4	
	55.9 + 19.3	56.7 + 14.2	50.7 + 23.5	
	62.0 + 21.1	59.9 + 19.2	57.2 + 18.7	
	68.6 + 16.6	72.6 + 24.9	75.3 + 14.8	
-2	84.4 + 10.4	88.2 + 2.7	80.9 + 23.7	0.375%
	84.9 + 11.4	88.6 + 3.5	80.2 + 22.2	
	82.7 + 15.7	83.7 + 6.8	76.9 + 16.2	
	84.7 + 17.2	78.3 + 10.6	83.3 + 12.7	
	86.2 + 10.1	86.2 + 13.9	88.9 + 8.0	

^a %Hemolysis, mean + S.D., N = 6 dogs/group (3 male, 3 female)

^b Significant difference between test and control at p < 0.05

Organ weights of male and female rats are also shown in Table 13. The only exposed organ group showing a dose related difference from controls was the male rat kidney which was significantly heavier in animals exposed at the higher level. The mean organ weights of the test female rats compared favorably with the control group of female rats.

TABLE 13. THE EFFECT OF 90-DAY CONTINUOUS EXPOSURE TO PETROLEUM JP-4 VAPOR ON ORGAN WEIGHTS

<u>Dogs</u>	<u>Unexposed Controls</u>	<u>Exposed 500 mg/m³</u>	<u>Exposed 1000 mg/m³</u>
Body weight, kg	11.15 \pm 1.46	13.58 \pm 3.10	11.67 \pm 2.21
Liver wt., gms	367.5 \pm 33.3	463.6 \pm 167.1	385.2 \pm 118.0
Liver/100 g body wt.	3.33 \pm 0.39	3.35 \pm 0.52	3.26 \pm 0.58
Spleen wt., gms	120.3 \pm 44.2	61.2 ^a \pm 33.2	58.4 ^a \pm 34.2
Spleen/100 g body wt.	1.08 \pm 0.28	0.45 ^b \pm 0.22	0.52 ^b \pm 0.33
Kidney wt., gms	5.6 \pm 0.9	5.8 \pm 1.5	4.9 \pm 1.0
Kidney/100 g body wt.	0.05 \pm 0.01	0.04 \pm 0.01	0.04 \pm 0.01
<u>Male Rats</u>			
Body weight, gm	324.3 \pm 17.8	306.0 ^b \pm 16.4	309.6 ^b \pm 21.0
Liver wt., gms	8.30 \pm 0.66	7.57 ^b \pm 0.75	8.08 \pm 0.91
Liver/100 g body wt.	2.56 \pm 0.11	2.48 \pm 0.22	2.61 \pm 0.18
Spleen wt., gms	0.55 \pm 0.05	0.57 ^b \pm 0.06	0.58 ^b \pm 0.08
Spleen/100 g body wt.	0.17 \pm 0.02	0.19 ^b \pm 0.02	0.19 ^b \pm 0.02
Kidney wt., gms	2.04 \pm 0.14	2.11 ^b \pm 0.22	2.31 ^b \pm 0.20
Kidney/100 g body wt.	0.63 \pm 0.03	0.69 ^b \pm 0.07	0.75 ^b \pm 0.06
<u>Female Rats</u>			
Body weight, gm	172.1 \pm 12.8	166.8 \pm 8.9	164.8 ^a \pm 7.2
Liver wt., gms	4.30 \pm 0.54	4.10 \pm 0.38	4.24 \pm 0.26
Liver/100 g body wt.	2.50 \pm 0.20	2.46 \pm 0.13	2.57 \pm 0.15
Spleen wt., gms	0.37 \pm 0.05	0.34 \pm 0.04	0.37 \pm 0.04
Spleen/100 g body wt.	0.21 \pm 0.02	0.21 \pm 0.02	0.22 \pm 0.02
Kidney wt., gms	1.24 \pm 0.15	1.22 \pm 0.12	1.24 \pm 0.08
Kidney/100 g body wt.	0.72 \pm 0.06	0.73 \pm 0.05	0.76 \pm 0.05

^a Significant, test vs. control, p < 0.05

^b Significant, test vs. control, p < 0.01

Gross tissue examination of the dogs revealed that 10 of 12 test dogs had roundworm infestation while only 1 of 6 control dogs exhibited this condition.

Exposure to JP-4 vapor affected the body weight gains of both the male and the female rats (Figure 9). The differences were noticeable throughout the exposure period and through three months postexposure in the male rats. The female rats recovered following exposure, and both test groups now show greater mean body weights than the controls.

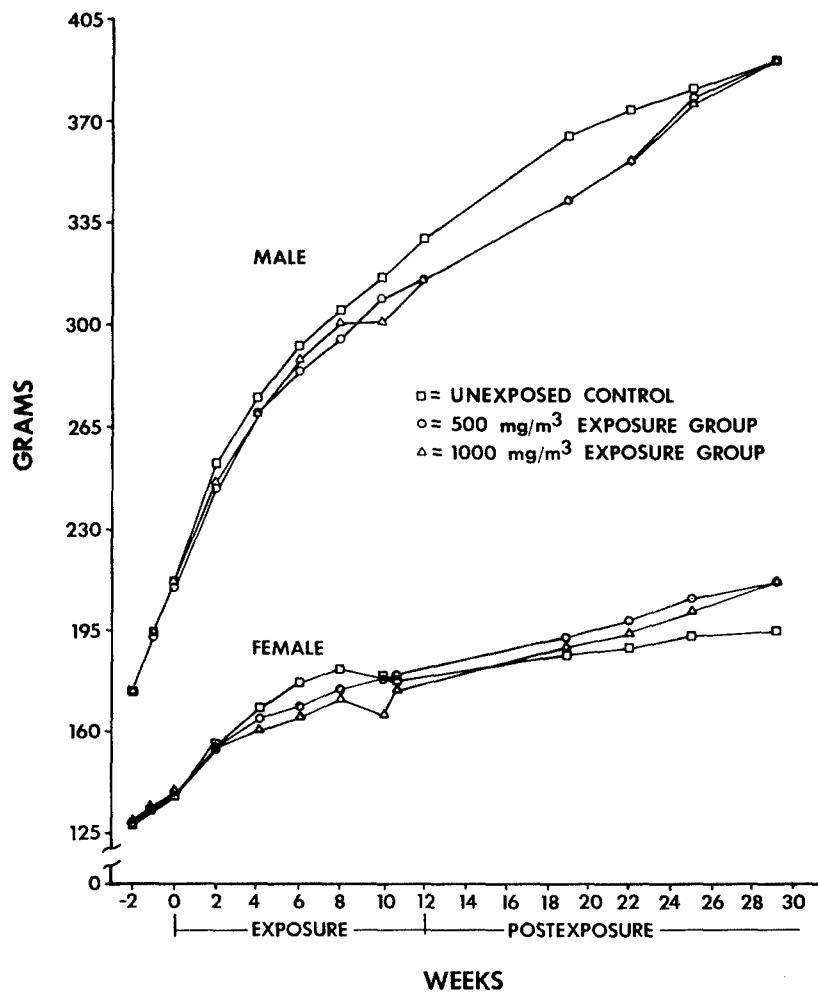


Figure 9. Effect of 90-Day continuous inhalation exposure to petroleum JP-4 vapor on rat weight.

The hematologic and clinical chemistry values of the male rats sacrificed at the conclusion of the 90-day exposure period are shown in Table 14. Slight, but significant, differences were found in several parameters; however, all but the creatinine values are within normal limits for male Fischer 344 rats. The values for female rats appear to be well within normal limits as shown in Table 15.

The remaining animals are being held postexposure in laminar flow animal facilities until February 1981.

TABLE 14. MEAN HEMATOLOGY AND CLINICAL CHEMISTRY
VALUES OF MALE RATS IMMEDIATELY AFTER 90-DAY
CONTINUOUS EXPOSURE TO PETROLEUM JP-4 VAPOR

	<u>Control</u>	<u>N</u>	<u>0.5 mg/liter</u>	<u>N</u>	<u>1.0 mg/liter</u>	<u>N</u>
RBC (10^6)	9.2	23	8.3	25	8.1 ^b	25
WBC (10^3)	6.7	24	6.3	25	5.7 ^a	25
HCT (%)	44.8	24	42.2	25	42.1	25
HGB (gm/dl)	15.5	23	14.9	25	14.6	25
Total Pro. (gm/dl)	7.0	11	6.9	8	7.1	16
Albumin (gm/dl)	3.8	11	3.6	8	3.7	16
Globulin (gm/dl)	3.2	11	3.3	8	3.4	16
A/G Ratio	1.2	11	1.1	8	1.1	16
Glucose (mg/dl)	114.3	11	110.9	8	103.8	16
Potassium (mEq/L)	5.3	11	5.1	8	5.4	16
Calcium (mg/dl)	10.2	11	10.1	8	10.3	16
Sodium (mEq/L)	149.5	11	152.8	8	149.9	16
Bilirubin (mg/dl)	0.47	11	0.50	8	0.49	16
Creatinine (mg/dl)	0.58	11	0.69	7	0.69	14
SGPT (IU/L)	47.3	11	45.0	8	47.3	16
SGOT (IU/L)	79.3	11	81.7	7	80.9	14
Alk. Phos. (IU/L)	9.8	11	9.4	8	8.7	16
BUN (mg/dl)	14.8	11	16.9	8	16.1	15
MCV	48.9	24	51.4	25	52.3	25
MCH	17.0	23	18.1	25	18.1	25
MCHC	34.7	23	35.2	25	34.7	25

^a - Significantly different from controls, $p < 0.05$.

^b - Significantly different from controls, $p < 0.01$.

TABLE 15. MEAN HEMATOLOGY AND CLINICAL CHEMISTRY
VALUES OF FEMALE RATS IMMEDIATELY AFTER 90-DAY
CONTINUOUS EXPOSURES TO PETROLEUM JP-4 VAPOR

	<u>Unexposed Controls</u>	<u>N</u>	<u>Exposed 500 mg/m³</u>	<u>N</u>	<u>Exposed 1000 mg/m³</u>	<u>N</u>
RBC (10^6)	7.6	24	7.7	24	7.6	23
WBC (10^3)	5.4	24	4.3	24	4.0	23
HCT (%)	41.8	24	41.2	24	40.9	23
HGB (gm/dl)	14.0	24	13.9	24	13.7	23
Total Pro. (gm/dl)	7.1	3	7.4	8	7.6	7
Albumin (gm/dl)	4.0	3	4.1	8	4.2	7
Globulin (gm/dl)	3.2	3	3.4	8	3.4	7
A/G Ratio	1.3	3	1.2	8	1.2	7
Glucose (mg/dl)	110.7	3	88.4	8	98.9	7
Potassium (mEq/L)	4.2	3	4.7	8	4.9	7
Calcium (mg/dl)	10.2	3	10.5	8	10.5	7
Sodium (mEq/L)	150.0	3	151.3	8	148.6	7
Bilirubin (mg/dl)	0.48	1	0.49	6	0.49	5
Creatinine (mg/dl)	0.67	1	0.60	3	0.60	3
SGPT (IU/L)	88.0	3	96.8	8	43.4	7
SGOT (IU/L)	132.0	1	76.0	2	73.0	2
Alk. Phos. (IU/L)	6.5	3	12.3	8	4.7	7
BUN (mg/dl)	18.0	1	16.8	5	14.3	3
MCV	55.1	24	53.8	24	53.9	23
MCH	18.4	24	18.2	24	18.1	23
MCHC	33.4	24	33.8	24	33.5	23

^a - Significantly different from controls, $p < 0.05$.

^b - Significantly different from controls, $p < 0.01$.

EVALUATION OF THE TOXIC EFFECT OF A 90-DAY CONTINUOUS
EXPOSURE TO PETROLEUM JP-5 VAPOR

A 90-day continuous inhalation toxicity study of JP-5 jet fuel vapors was conducted by the Toxic Hazards Research Unit during 1977 as the first in a series of subchronic exposures to hydrocarbon fuels. Detailed discussions of the protocol, contaminant generation, and monitoring system were presented in previous annual reports (MacEwen and Vernot, 1978, 1979). Since the last report, the interim and final rodent sacrifices have occurred. This report updates and summarizes results of the study which were not available for previous reports.

As is standard procedure in 90-day continuous subchronic studies, groups of 3 male and female beagle dogs, 75 male and female Fischer 344 rats, and 150 female C57B1/6 mice were continuously exposed to concentrations of 150 mg/m³ or 750 mg/m³ petroleum JP-5 vapors for 90 days in Thomas Dome inhalation chambers. Unexposed controls were held in laminar air flow rooms in separate facilities. At the conclusion of the exposure, all dogs and 1/3 of the rodents were sacrificed for gross and histopathologic tissue examination to detect any pathologic lesions caused by exposure to petroleum JP-5.

The remaining rodents were held for postexposure observation for 19 months. At that time, one-half of the rats were sacrificed for tissue collection and examination. Animals remaining from this interim sacrifice were held until the 24th month of the study at which time all surviving animals were sacrificed for tissue examination. This final sacrifice occurred in June 1979.

The body weights of male and female rats are shown in Figures 10 and 11, respectively. The weights of male rats exposed to 750 mg/m³ petroleum JP-5 vapor were significantly ($p < 0.01$) less than unexposed control male rats through the exposure and postexposure phases of the study. Male rats exposed to 150 mg/m³ petroleum JP-5 vapor also weighed significantly ($p < 0.01$) less than unexposed control rats. However, this difference continued only through the 16th month of the study at which time the weights of this exposure group returned to the level of the unexposed control rats. Exposure to petroleum JP-5 vapor had no effect on the body weight gain of female rats.

Blood samples were obtained from all rats sacrificed at 19-months postexposure. The hematologic and clinical chemistry values of male rats are shown in Table 16. Slight, but statistically significant, elevations were noted in globulin and creatinine levels of male rats exposed to 750 mg/m³ petroleum JP-5. There was also a slight increase in BUN levels of the male rats exposed to petroleum JP-5 vapors when compared to unexposed controls. The increased BUN and creatinine in male rats may have been an indication of some form of renal damage.

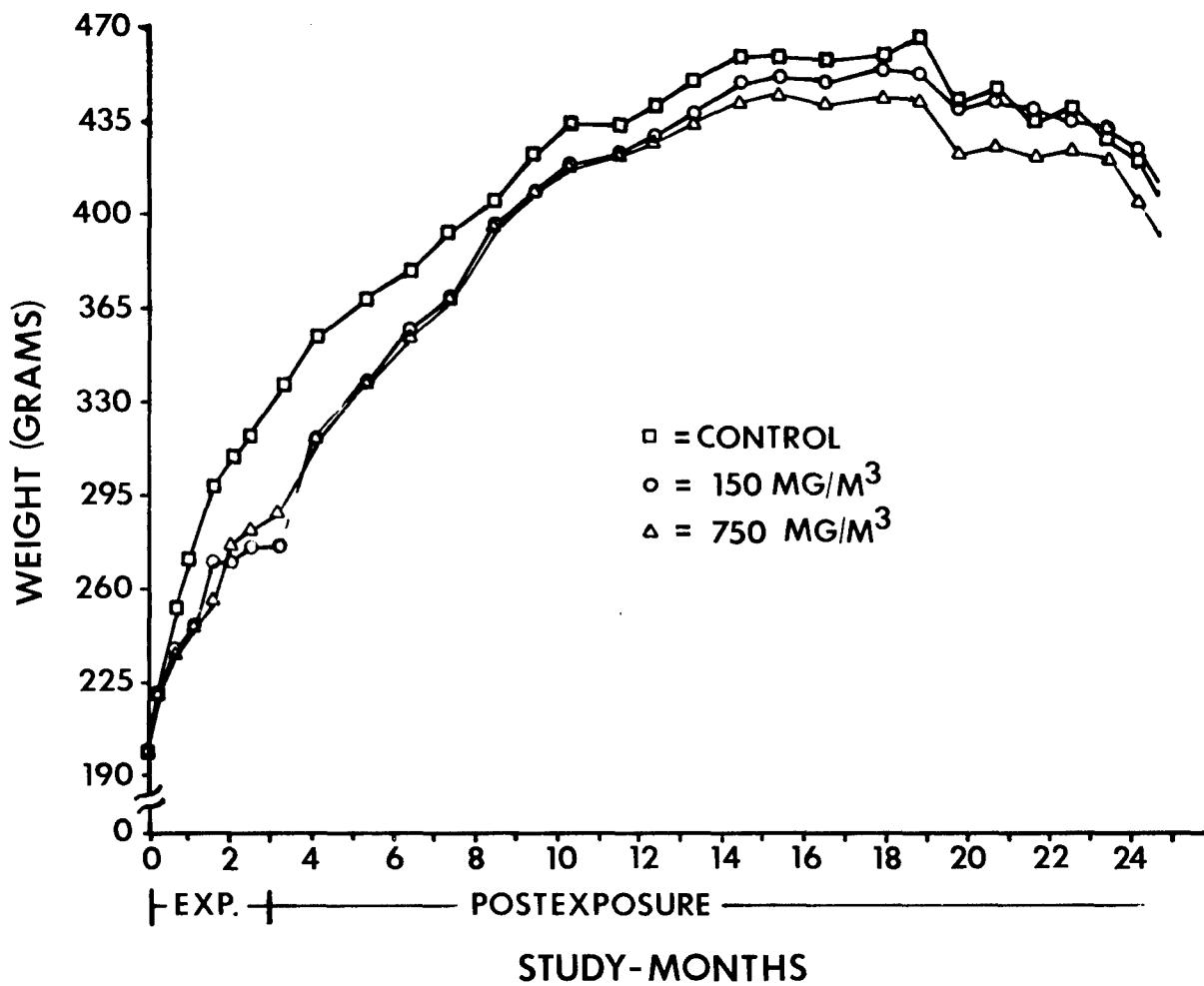


Figure 10. Effect of 90-day continuous inhalation exposure to petroleum JP-5 vapor on male rat body weight.

The values presented in Table 16 exclude results obtained from three control male rats with WBC counts of 48,000 cells per mm^3 or greater. In these rats, 80-90% of the WBC's were leukemic mononuclear cells with one of the rats also showing increased levels of alkaline phosphatase (272.8 IU/liter) and SGOT (1054 IU/liter). Abnormally high WBC counts or leukemic mononuclear cells were not seen in any of the rats exposed to petroleum JP-5. Goodman et al. (1979) have found lymphomas and leukemias of all types to be among the most common tumors in older Fischer 344 male and female rats.

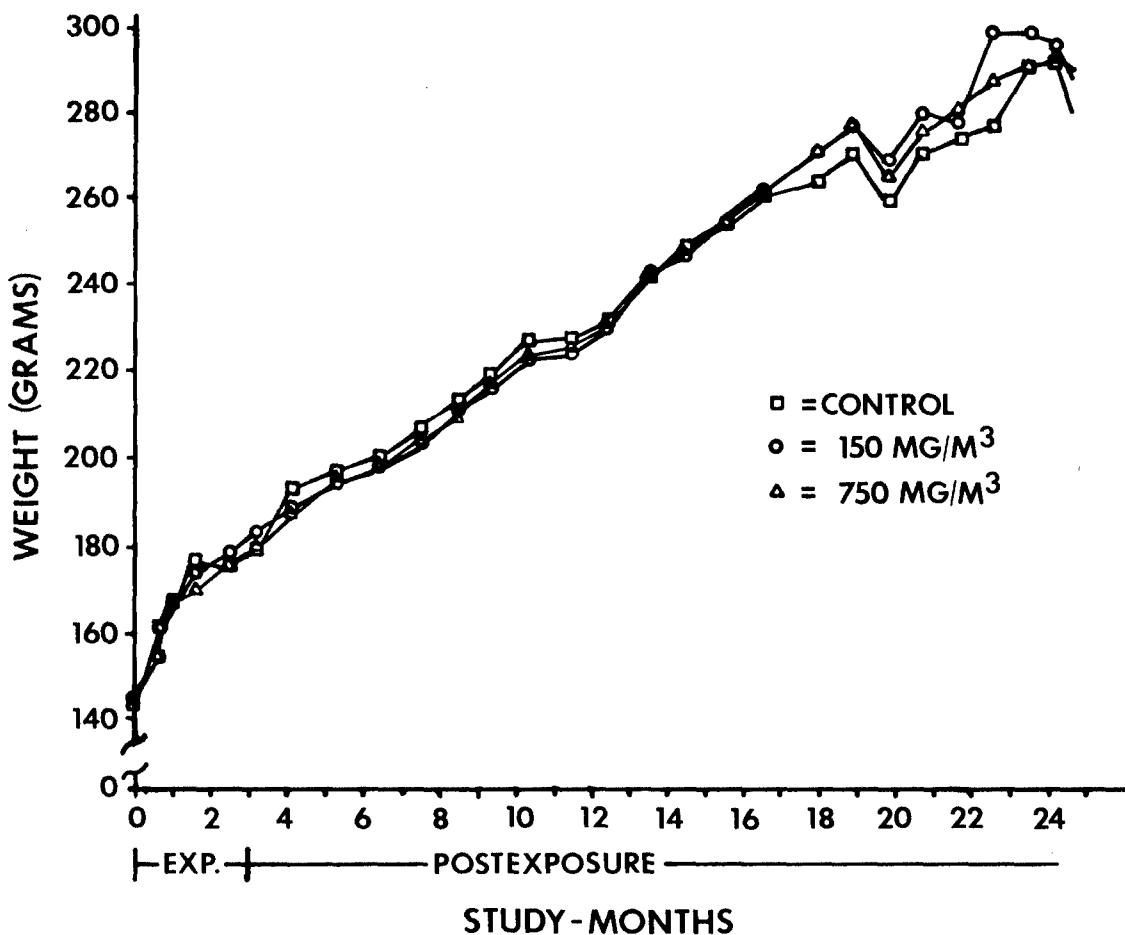


Figure 11. Effect of 90-day continuous inhalation exposure to petroleum JP-5 vapor on female rat body weight.

Three male rats from the 150 mg/m^3 petroleum JP-5 exposure group and 3 male rats from the 750 mg/m^3 petroleum JP-5 exposure group had RBC values greater than 10×10^6 . These values were not determined exactly since the Hycel-300 used to determine the RBC count has an upper detection limit of 10×10^6 , and no dilution of the sample was attempted at the time of testing. The values from these rats were, therefore, excluded from any RBC statistical analysis.

Mean SGPT and SGOT values of the male rats exposed to 150 mg/m^3 petroleum JP-5 were greater than unexposed male control rats because of one rat that had an SGPT value of 834 IU/liter and SGOT value of 826 IU/liter. Since the elevations of the SGPT and SGOT means were due primarily to one sample value, there was no statistically significant difference between control and exposure groups.

TABLE 16. MEAN HEMATOLOGY AND CLINICAL CHEMISTRY VALUES OF MALE RATS 19 MONTHS AFTER 90-DAY CONTINUOUS EXPOSURE TO PETROLEUM JP-5 VAPOR

	Control	N	150 mg/m ³	N	750 mg/m ³	N
RCB (10 ⁶)	8.7	14	8.5 ^c	14	7.9 ^c	18
WBC (10 ³)	6.6 ^d	14	7.2	17	7.7	21
HCT (%)	50.7	14	51.5	17	50.5	21
HGB (gm/dl)	16.2	14	16.7	17	16.5	21
Total Pro. (gm/dl)	7.4	10	7.1	12	7.3	14
Albumin (gm/dl)	4.2	10	4.0	12	3.8 ^a	14
Globulin (gm/dl)	3.1	10	3.1	12	3.5 ^a	14
A/G ratio	1.4	10	1.3	12	1.1 ^b	14
Glucose (mg/dl)	118.8	10	111.3	12	114.1	14
Potassium (mEq/L)	5.2	10	5.4	12	5.7	15
Calcium (mg/dl)	10.9	10	10.6	12	11.2	14
Sodium (mEq/L)	157.9	10	155.8	12	157.9	15
Bilirubin (mg/dl)	0.67	10	0.70	12	0.66	9
BUN (mg/dl)	16.6	10	17.6	12	18.7	10
Creatinine (mg/dl)	0.61	10	0.63	12	0.80 ^a	9
SGPT (IU/L)	55.8	10	124.2 ^e	12	44.6	13
SGOT (IU/L)	90.6	10	156.5 ^e	12	74.2	10
Alk. Phos. (IU/L)	13.4	10	9.0 ^b	12	7.2 ^b	10

^a Significantly different from controls, $p < 0.05$.

^b Significantly different from controls, $p < 0.01$.

^c 3 samples $> 10 \times 10^6$ omitted.

^d 3 samples with leukemia cells omitted.

^e High mean due to one value, not significantly different from controls.

The hematological and clinical chemistry values of female rats sacrificed at 19-months postexposure are shown in Table 17. The exposure groups are significantly different from the unexposed control group in a number of parameters. However, the values are still within normal limits and differences noted are probably not exposure related.

Organ weights of the rats sacrificed at 19 months postexposure are shown in Tables 18 and 19 for male and females, respectively.

The spleens of three unexposed control male rats were unusually large (42, 39, and 17 grams). These rats were those with leukemic mononuclear cells described earlier. The remaining control spleen weights were comparable to those of the exposed rats. The kidneys of the male rats exposed to 750 mg/m³ petroleum JP-5 vapors were heavier than kidneys of the unexposed controls. The abnormal parameters associated with kidney damage in male rats exposed to 750 mg/m³ petroleum JP-5 at 19 months postexposure may indicate that the observed deleterious effects are not reversible after removal from continuous exposure.

TABLE 17. MEAN HEMATOLOGY AND CLINICAL CHEMISTRY VALUES OF FEMALE RATS 19 MONTHS AFTER 90-DAY CONTINUOUS EXPOSURE TO PETROLEUM JP-5 VAPOR

	<u>Control</u>	<u>N</u>	<u>150 mg/m³</u>	<u>N</u>	<u>750 mg/m³</u>	<u>N</u>
RCB (10 ⁶)	7.9	13	8.5 ^a	17	8.8 ^b	20
WBC (10 ³)	5.4	13	4.4	18	4.4	20
HCT (%)	44.3	13	45.4	18	45.1	20
HGB (gm/dl)	14.7	13	15.0	18	14.9	20
Total Pro. (gm/dl)	7.6	5	7.7	10	7.7	14
Albumin (gm/dl)	4.2	5	4.3	10	4.2	14
Globulin (gm/dl)	3.4	5	3.4	10	3.4	14
A/G ratio	1.2	5	1.3	10	1.2	14
Glucose (mg/dl)	121.6	5	116.4	10	138.1	14
Potassium (mEq/L)	5.5	5	5.9	10	6.1 ^a	14
Calcium (mg/dl)	10.7	5	10.1 ^a	10	10.0 ^b	14
Sodium (mEq/L)	147.8	5	149.9 ^a	10	148.6	14
Bilirubin (mg/dl)	0.51	5	0.48	10	0.43	14
BUN (mg/dl)	15.0	5	15.5	10	15.5	14
Creatinine (mg/dl)	0.60	5	0.58	10	0.49 ^a	14
SGPT (IU/L)	51.6	5	52.6	10	46.1	14
SGOT (IU/L)	121.2	5	111.0	10	97.7	14
Alk. Phos. (IU/L)	7.8	5	6.7	10	6.4	14

^a Significantly different from controls, p < 0.05.

^b Significantly different from controls, p < 0.01.

TABLE 18. MALE RAT ORGAN WEIGHTS^a MEASURED 19 MONTHS AFTER 90-DAY CONTINUOUS EXPOSURE TO PETROLEUM JP-5

	<u>Control</u> (N=17)	<u>150 mg/m³</u> (N=17)	<u>750 mg/m³</u> (N=17)
Body weight, gm	419.5 \pm 29.7	420.1 \pm 29.3	397.7 \pm 19.9 ^b
Liver wt., gms	12.4 \pm 2.2	12.2 \pm 1.1	12.5 \pm 1.6
Liver/100 g body wt.	2.98 \pm 0.70	2.90 \pm 0.23	3.16 \pm 0.43
Kidney wt., gms	2.8 \pm 0.2	2.9 \pm 0.2	3.0 \pm 0.3 ^b
Kidney/100 g body wt.	0.68 \pm 0.09	0.67 \pm 0.04	0.77 \pm 0.09 ^b
Spleen wt., gms	6.5 \pm 12.6	1.6 \pm 1.5	1.4 \pm 0.7
Spleen/100 g body wt.	1.63 \pm 3.17	0.39 \pm 0.59	0.35 \pm 0.18

^a Mean \pm S.D.

^b Significantly different from control, p < 0.05.

TABLE 19. FEMALE RAT ORGAN WEIGHTS^a MEASURED 19 MONTHS AFTER 90-DAY CONTINUOUS EXPOSURE TO PETROLEUM JP-5

	Control (N=17)	150 mg/m ³ (N=17)	750 mg/m ³ (N=17)
Body weight, gm	268.2 \pm 26.6	269.8 \pm 21.6	273.2 \pm 21.5
Liver wt., gms	6.8 \pm 0.7	6.8 \pm 0.9	6.8 \pm 0.6
Liver/100 g body wt.	2.54 \pm 0.21	2.51 \pm 0.23	2.48 \pm 0.21
Kidney wt., gms	1.8 \pm 0.3	1.8 \pm 0.2	1.7 \pm 0.3
Kidney/100 g body wt.	0.67 \pm 0.06	0.66 \pm 0.05	0.63 \pm 0.13
Spleen wt., gms	0.77 \pm 0.48	0.53 \pm 0.17	0.70 \pm 0.39
Spleen/100 g body wt.	0.29 \pm 0.17	0.19 \pm 0.06	0.26 \pm 0.15

^a Mean \pm S.D.

Histopathologic examinations of the animal tissues taken in this study are not complete. Therefore, the indications of permanent kidney damage in petroleum JP-5 exposed male rats cannot be confirmed at this time.

A SUBCHRONIC INHALATION TOXICITY STUDY OF A 90-DAY CONTINUOUS INHALATION EXPOSURE TO SHALE JP-5/AVIATION GAS MIXTURE

A companion study to the petroleum JP-5 fuel subchronic toxicity study was initiated for the inhalation of JP-5 fuel vapor of shale oil origin. This study involves the exposure of groups of three male and female beagle dogs, 75 male and 75 female Fischer 344 rats, and 150 female C57B1/6 mice to vapor of the jet fuel JP-5 refined from Colorado oil shale. A similar number of unexposed control animals was housed in laminar airflow facilities in a separate building. The concentrations of shale JP-5 used were 150 mg/m³ and 750 mg/m³. These were the same as the concentrations used in the previous study of petroleum JP-5.

The shale JP-5 vapor generation and analysis system used resembled the design of the system used in the generation of petroleum JP-5 (MacEwen and Vernot, 1978). The experimental protocol followed was also similar to that used in the petroleum JP-5 study.

Rats and dogs were weighed individually at biweekly intervals during exposure and rats monthly during the postexposure period. Mice were weighed in groups and group mean weights followed on a monthly basis throughout the experimental period. Blood samples were drawn from dogs at biweekly intervals and clinical determinations made for the same series of tests shown in Table 11 for JP-4.

Gas chromatographic analysis of samples of the first five drums of shale JP-5 used during the initial period of the exposure indicated that all five drums contained virtually identical materials. A significant portion of the material consisted of low boiling components including toluene. Gas chromatographic analysis of subsequent deliveries of drums of shale JP-5 revealed that the material in the drums differed significantly from that seen in the first five drums. The later drums contained hardly any of the low boiling compounds present in the earlier samples. Analysis of all the remaining drums of shale JP-5 to be used in the study revealed that only 6 contained the low boiling materials found in the initial drums.

The shale JP-5 used in the study was from a 100,000 barrel refinement of Colorado oil shale. A portion of this refinement was sent via rail tank car to Rickenbacker Air Force Base, Ohio, where it was placed in a storage tank. From this point, 55 gallon drums were filled and sent to the THRU for the inhalation test. Since the storage tank had previously held aviation gas, it was cleaned and rinsed prior to filling with shale JP-5. However, there was apparently a section of the tank or associated piping that was not cleaned, thus allowing for some contamination of the shale JP-5.

There was not enough of the shale JP-5/aviation gas mixture to continue the study for the scheduled 90-day period. Continuing the exposure with uncontaminated material, thereby altering the consistency of the exposure, was an unacceptable alternative. Therefore, the exposures were terminated at 60 days of exposure. All dogs and 1/3 of the mice were sacrificed for tissue examination. The remaining mice are being held for postexposure observation. Because of a staggered exposure initiation schedule for individual species typically used by the THRU in the 90-day continuous exposure studies, a full 60-day exposure of rats could not be completed. All rats were terminated and no biological measurements were recorded.

Gas chromatographic analysis showed that the bulk shale JP-5 used from the 5 barrels was contaminated with 3-5% aviation gas which comprised about 30% of the total fuel vapor in the exposure chambers. Hydrocarbon concentration in the exposure chambers was continuously measured with a Beckman 400 hydrocarbon analyzer. Mean and standard deviations for the exposures were 150.2 ± 2.6 mg/m³ and 752.5 ± 13.6 mg/m³.

Body weights of beagle dogs were unaffected by exposure to shale JP-5/aviation gas. Blood parameters of exposed dogs remained within normal limits through the exposure. Red blood cell osmotic fragility was tested in dog blood. The results are shown in Table 20. Exposure to shale JP-5/aviation gas had little effect on RBC fragility. Organ weights of dogs were measured at the conclusion of the exposure and are presented in Table 21.

TABLE 20. EFFECT OF 60-DAY CONTINUOUS EXPOSURE TO
SHALE JP-5/AVIATION GAS ON DOG RED BLOOD CELL FRAGILITY^a

Exposure Weeks	Control	500 mg/m ³	1000 mg/m ³	Saline Conc.
-2	4.8 + 0.7	11.2 + 7.7 ^b	5.8 + 3.4	0.50%
	3.3 + 1.5	6.7 + 3.5	3.4 + 2.3	
	5.3 + 1.2	17.3 + 3.5	11.4 + 7.2 ^b	
	2.7 + 1.2	7.7 + 4.6 ^b	6.8 + 2.6 ^b	
	5.7 + 2.9	8.5 + 2.9	5.0 + 1.9	
0	12.9 + 2.9	36.9 + 23.1 ^b	15.6 + 9.6	0.475%
	9.1 + 3.4	20.9 + 11.1 ^b	11.9 + 6.2	
	13.4 + 3.3	35.9 + 10.4 ^b	23.1 + 11.4	
	10.3 + 6.8	13.3 + 12.1	16.1 + 10.1	
	10.2 + 3.8	20.1 + 6.0 ^b	13.5 + 4.4	
4	29.4 + 3.9	43.9 + 22.7 ^b	29.2 + 6.5	0.45%
	23.7 + 9.2	44.6 + 17.8 ^b	26.9 + 5.7	
	35.9 + 7.8	67.9 + 11.4 ^b	51.8 + 15.5 ^b	
	22.5 + 13.6	39.4 + 15.4 ^b	38.4 + 12.8 ^b	
	26.8 + 7.0	46.7 + 10.7 ^b	32.6 + 10.5	
6	53.1 + 9.7	67.5 + 21.0 ^b	49.7 + 18.9	0.425%
	46.8 + 11.8	61.5 + 16.5 ^b	47.3 + 16.6 ^b	
	58.5 + 13.7	87.3 + 7.1 ^b	75.1 + 12.6 ^b	
	56.2 + 20.9	66.6 + 17.9	56.5 + 28.6	
	49.5 + 10.3	68.5 + 8.8 ^b	55.8 + 14.6	
8	80.1 + 7.6	86.1 + 4.1	78.1 + 5.6	0.40%
	73.1 + 10.8	83.9 + 10.2	70.7 + 16.2	
	84.9 + 5.3	96.2 + 2.3	91.5 + 5.8 ^b	
	78.6 + 12.5	90.4 + 2.6 ^b	84.4 + 11.4	
	77.4 + 7.1	91.1 + 3.0 ^b	81.8 + 10.0	
-2	93.4 + 2.9	95.4 + 2.8	91.1 + 5.4	0.375%
	90.4 + 4.4	94.4 + 3.2	86.3 + 10.7	
	94.6 + 1.8	98.1 + 1.5 ^b	97.4 + 2.3 ^b	
	89.7 + 6.2	93.9 + 3.4	94.5 + 4.9	
	88.9 + 4.1	96.6 + 1.3 ^b	92.5 + 4.9	

^a % hemolysis, mean + S.D., N=6 day/gram, 3 male, 3 female

^b Significantly different from control, p < 0.05.

The spleen weights of the dogs exposed to 750 mg/m³ shale JP-5/aviation gas were statistically less than unexposed controls. No dose related effect is indicated since the mean spleen weight of the dogs exposed to 150 mg/m³ shale JP-5/aviation gas is slightly larger than unexposed controls.

TABLE 21. EFFECT OF 60-DAY CONTINUOUS EXPOSURE
TO SHALE JP-5/AVIATION GAS ON ORGAN WEIGHTS OF BEAGLE DOGS

	<u>Control</u>	<u>150 mg/m³</u>	<u>750 mg/m³</u>
Body weight, kg	9.52 \pm 1.55	11.02 \pm 2.79	10.23 \pm 1.35
Liver wt., gms	329.8 \pm 67.1	352.7 \pm 70.3	384.9 \pm 47.4
Liver/100 g body wt.	3.4 \pm 0.3	3.3 \pm 0.6	3.8 \pm 0.5
Kidney wt., gms	48.2 \pm 10.8	49.9 \pm 13.4	47.9 \pm 13.2
Kidney/100 g body wt.	0.50 \pm 0.006	0.46 \pm 0.05	0.46 \pm 0.07
Spleen wt., gms	72.1 \pm 35.6	105.1 \pm 54.3	43.0 \pm 10.1
Spleen/100 g body wt.	0.75 \pm 0.31	0.95 \pm 0.39	0.42 \pm 0.07 ^a

^a Significantly different from controls p < 0.05.

Histopathologic examination of the tissues from the dogs and mice sacrificed at the conclusion of the 60-day exposure is not complete at this time.

A SUBCHRONIC TOXICITY STUDY OF A 90-DAY CONTINUOUS INHALATION EXPOSURE TO SHALE JP-5 VAPOR

Because of the contamination of the shale JP-5 and subsequent early termination of the exposure, it was decided to rerun the study. All drums of shale JP-5 available for use were examined for possible contamination by aviation gas. An adequate supply of shale JP-5, essentially free of aviation gas, was available for the completion of a 90-day exposure. A new supply of animals was obtained. The animal group size remained at 3 male, 3 female beagle dogs, 75 male and 75 female Fischer 344 rats, and 150 female C57B1/6 mice per exposure and control group. Mean shale JP-5 concentrations for the 90-day exposure were determined to be 150.4 \pm 1.3 mg/m³ and 750.5 \pm 5.2 mg/m³.

Continuous exposure to shale JP-5 vapor for 90 days had no effect on dog body weight. Blood parameters measured in dogs during the exposure were within normal limits expected for this species. Red blood cell osmotic fragility test results are shown in Table 22 and show that exposures to shale JP-5 vapor had no effect.

Dog organ weights were measured at the conclusion of the exposure. The results of these measurements are shown in Table 23.

TABLE 22. EFFECT OF 90-DAY CONTINUOUS EXPOSURE TO
SHALE JP-5 VAPOR ON RED BLOOD CELL FRAGILITY^a IN
BEAGLE DOGS

Exposure Weeks	Control	500 mg/m ³	1000 mg/m ³	Saline Conc.
-2	3.2 + 1.1	3.7 + 2.6	4.8 + 2.5	0.50%
0	4.4 + 1.1	4.8 + 1.8	7.3 + 5.7	
4	4.2 + 3.7	4.7 + 4.3	5.0 + 2.5	
8	1.9 + 1.2	3.9 + 1.3	4.5 + 2.1	
12	2.1 + 0.1	2.5 + 2.1	3.2 + 1.2	
-2	8.8 + 3.4	10.9 + 4.0	12.2 + 3.3	0.475%
0	8.5 + 1.3	10.2 + 3.9	12.3 + 9.7	
4	6.8 + 5.6	6.2 + 4.2	11.7 + 3.9	
8	5.2 + 2.1	7.1 + 2.4	12.4 + 6.5	
12	2.1 + 0.1	4.5 + 1.9	5.9 + 2.0	
-2	20.4 + 3.9	26.7 + 10.6	33.8 + 7.8	0.45%
0	16.2 + 2.9	21.9 + 8.4	25.9 + 16.8	
4	18.2 + 5.0	18.8 + 9.6	25.8 + 13.5	
8	14.0 + 3.3	20.5 + 6.9	28.7 + 12.2	
12	10.6 + 2.9	16.3 + 10.5	15.1 + 5.2	
-2	42.3 + 8.1	49.1 + 12.8	49.4 + 12.6	0.425%
0	39.9 + 4.9	53.7 + 12.4	51.9 + 17.0	
4	41.8 + 5.9	42.8 + 14.8	51.3 + 11.0	
8	28.9 + 8.1	35.2 + 12.9	43.4 + 10.4	
12	28.0 + 10.3	30.2 + 13.0	35.6 + 6.5	
-2	70.6 + 12.8	76.7 + 8.9	73.3 + 10.7	0.40%
0	69.2 + 5.4	79.6 + 8.9	78.6 + 8.6	
4	77.4 + 9.4	75.8 + 7.9	82.8 + 4.8	
8	59.8 + 6.3	66.5 + 15.0	78.1 + 6.4	
12	47.1 + 7.6	51.9 + 17.5	62.3 + 8.1	
-2	93.3 + 2.6	93.7 + 3.7	94.3 + 4.1	0.375%
0	86.6 + 3.3	90.1 + 4.2	92.8 + 3.7	
4	87.4 + 7.9	84.8 + 8.8	91.6 + 3.2	
8	84.5 + 3.9	85.2 + 9.9	93.9 + 2.5	
12	75.6 + 9.9	82.2 + 8.2	82.8 + 6.5	

^a % hemolysis, mean + S.D., N = 6 dogs/group
(3 male, 3 female)

The liver/body ratio of dogs exposed to 750 mg/m³ shale JP-5 vapor was greater than unexposed control dogs. The increase is not evident in dogs exposed to 150 mg/m³ shale JP-5. Histopathologic examination of the tissues obtained from the dogs was not completed for this report; therefore, it is not known if the increase in liver/body weight ratio in dogs exposed to 750 mg/m³ shale JP-5 is related to actual liver injury.

Gross pathologic examination of the dogs revealed that many had roundworm infestation. This finding was equally divided between male and female dogs.

TABLE 23. EFFECT OF 90-DAY CONTINUOUS EXPOSURE TO SHALE JP-5 VAPOR ON ORGAN WEIGHTS^a OF BEAGLE DOGS

	<u>Control</u>	<u>150 mg/m³</u>	<u>750 mg/m³</u>
Body weight, kg	10.02 \pm 1.32	11.56 \pm 1.44	11.68 \pm 2.18
Liver wt., gms	291.9 \pm 23.0	340.4 \pm 37.7	424.9 \pm 121 ^b
Liver/100 g body wt.	2.94 \pm 0.31	2.95 \pm 0.18	3.62 \pm 0.65 ^b
Spleen wt., gms	71.6 \pm 24.4	75.5 \pm 30.2	79.2 \pm 19.2
Spleen/100 g body wt.	0.72 \pm 0.24	0.66 \pm 0.27	0.69 \pm 0.24
Kidney wt., gms	51.2 \pm 6.8	51.5 \pm 5.3	57.9 \pm 9.7
Kidney/100 g body wt.	0.51 \pm 0.07	0.45 \pm 0.04	0.50 \pm 0.08

^a Mean \pm S.D., N=6

^b Significantly different from control, p < 0.05.

The body weights of both groups of male rats exposed to shale JP-5 vapor were significantly (p < 0.05) lower than unexposed control male rats after two weeks exposure (Figure 12). This trend continues 6 months postexposure with an apparent dose related response.

The body weights of the female rats exposed to 750 mg/m³ shale JP-5 were significantly (p < 0.05) less than unexposed controls after 2 weeks of exposure and remain less than controls 6 months postexposure (Figure 13). Weight differences between female rats exposed to 150 mg/m³ shale JP-5 and unexposed control female rats have been transient in nature with the most notable differences occurring during the middle of the 90-day exposure. By the conclusion of the exposure, the body weights of the female rats in the 150 mg/m³ exposure group had returned to control values and remained similar 6 months postexposure.

The hematology and clinical chemistry results obtained from rats at the conclusion of the 90-day continuous exposure to shale JP-5 are shown in Tables 24 and 25 for males and females, respectively. RBC, HCT, and HGB values of male rats exposed to 750 mg/m³ shale JP-5 vapor were lower than unexposed controls. However, corpuscular indices of shale JP-5 exposed male rats indicate that the red cell size, amount of hemoglobin per cell, and concentration of hemoglobin per cell were not different than unexposed male control rats. Some form of shale JP-5 induced kidney injury may be indicated by the increase in BUN and creatinine values of male rats exposed to 750 mg/m³ shale JP-5.

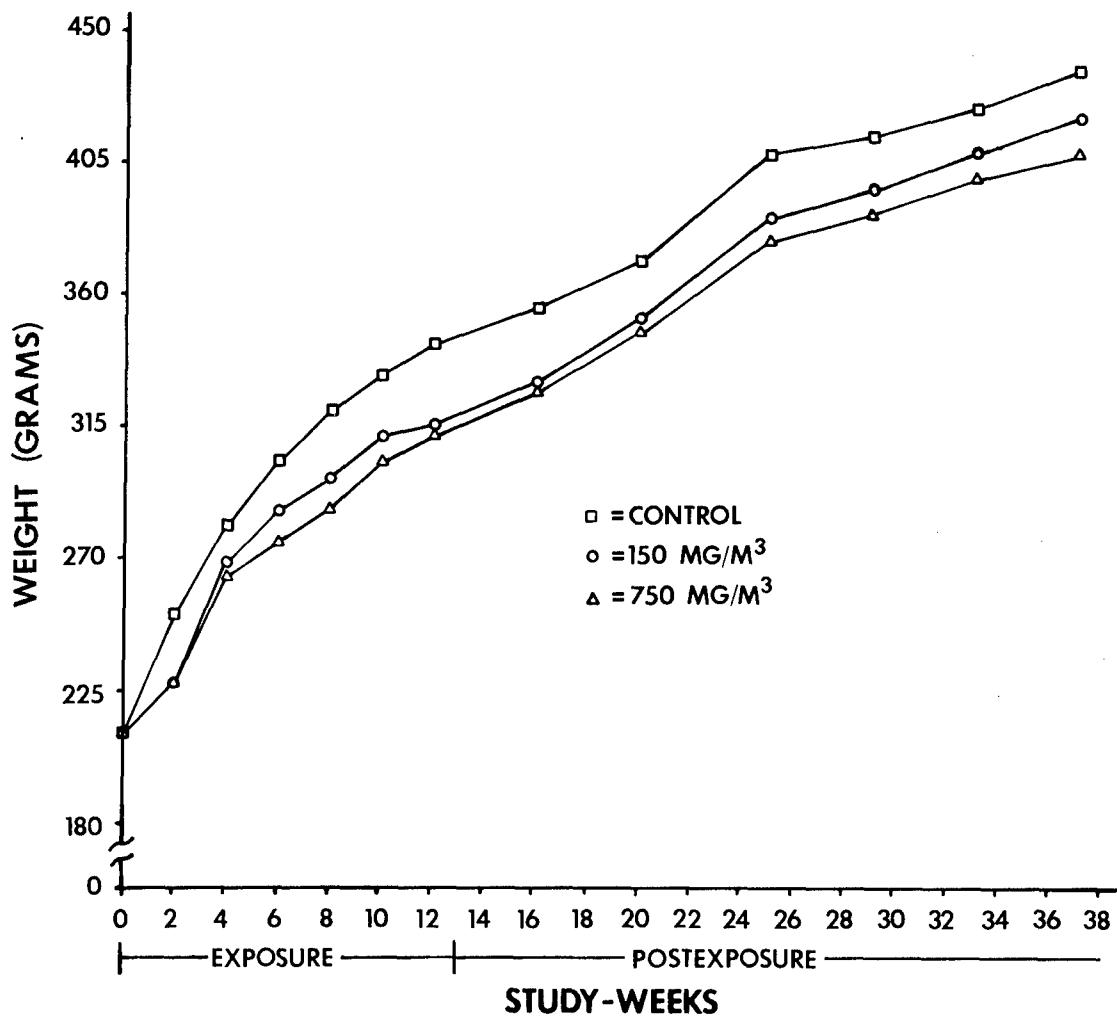


Figure 12. Effect of 90-day continuous inhalation exposure to shale JP-5 vapor on male rat body weight.

Similar increases in these two parameters occurred in male rats exposed to petroleum JP-5 vapor for 90 days (MacEwen and Vernot, 1978). Kidney injury was seen in those rats. There were a number of other parameters measured in male rats exposed to shale JP-5 that were statistically different from unexposed controls. The values were, however, within normal ranges for the species.

Female rats exposed to 750 mg/m³ shale JP-5 vapor had lower hematocrit and hemoglobin values when compared to their corresponding controls. Corpuscular indices of shale JP-5 exposed female rats were not different from the indices of unexposed female control rats. Other values of blood parameters measured in shale JP-5 exposed female rats, while statistically different from unexposed controls, were within normal ranges.

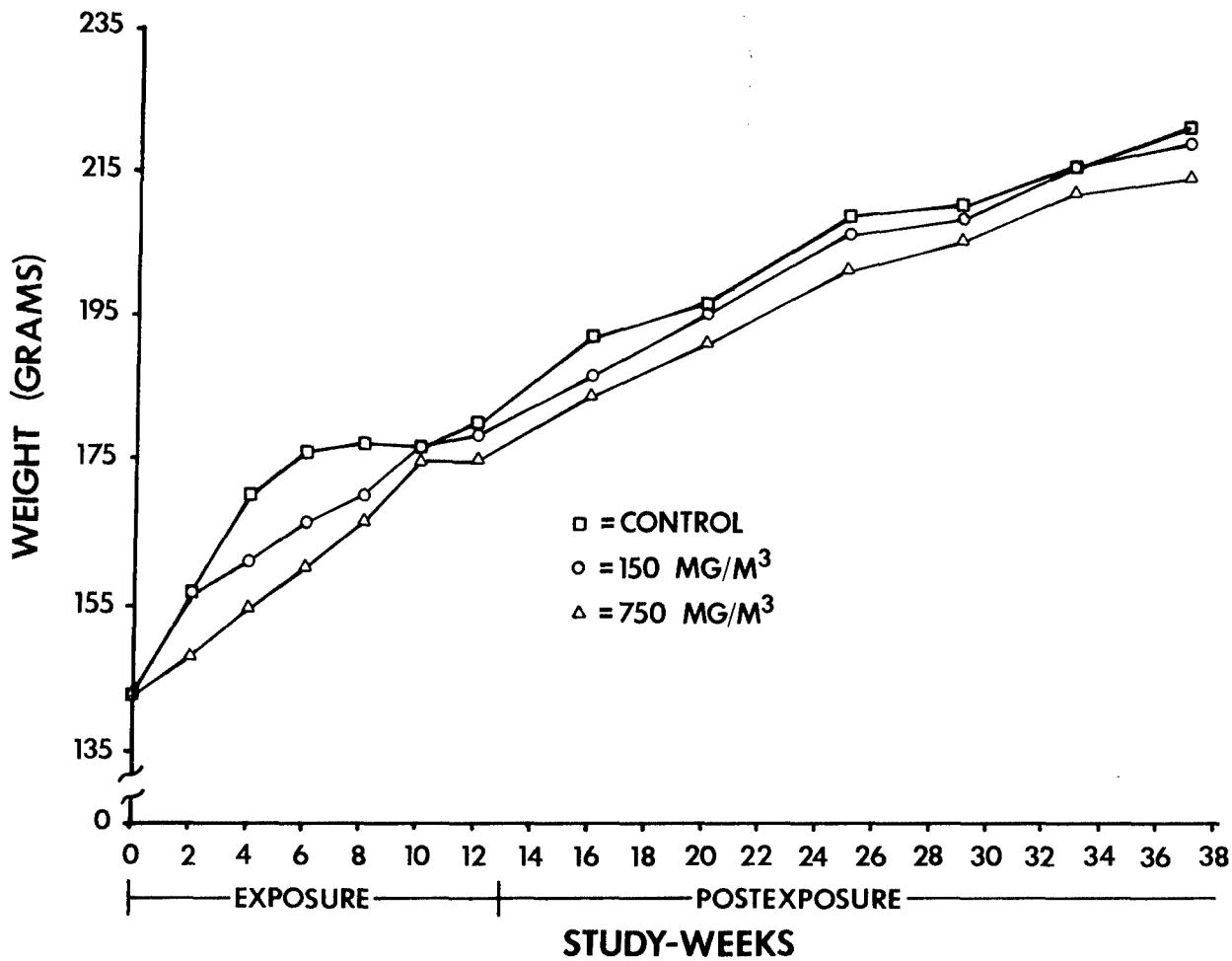


Figure 13. Effect of 90-day continuous inhalation exposure to shale JP-5 vapor on female rat body weight.

Rat organ weights measured at the conclusion of the exposure are shown in Tables 26 and 27 for male and female rats, respectively.

Increased liver, spleen, and kidney weights of the male and female rats exposed to 750 mg/m³ are indicated by the organ to body weight ratios for these groups. Since histopathologic examination of the tissues obtained at the conclusion of the exposure is not complete, it is not known if the increased organ/body weight ratios are indicative of actual tissue injury. There was no dose related effect on organ weights since the organ weights of rats exposed to 150 mg/m³ were less than unexposed controls.

Examination of the tissues collected from the dogs, rats, and mice at the conclusion of the exposure was not completed in time for this report. The study is continuing.

TABLE 24. HEMATOLOGY AND CLINICAL CHEMISTRY VALUES OF MALE RATS AFTER 90-DAY CONTINUOUS INHALATION EXPOSURE TO SHALE JP-5 VAPOR

	<u>Control</u>	<u>N</u>	<u>150 mg/m³</u>	<u>N</u>	<u>750 mg/m³</u>	<u>N</u>
RBC (10 ⁶)	9.8	25	9.6	25	9.1 ^a	25
WBC (10 ³)	6.3	25	6.5	25	6.8	25
HCT (%)	49.3	25	47.2 ^a	25	44.4 ^a	25
HGB (gm/dl)	16.3	25	15.9	25	15.0 ^a	25
MCV	50.1	25	49.2	25	49.1	25
MCH	16.6	25	16.6	25	16.6	25
MCHC	33.1	25	33.7	25	33.8	25
Total Pro. (gm/dl)	7.2	24	7.2	25	6.9 ^a	25
Albumin (gm/dl)	4.1	24	3.9 ^a	25	3.8 ^a	25
Globulin (gm/dl)	3.0	24	3.3 ^a	25	3.2 ^a	25
A/G Ratio	1.37	24	1.2 ^a	25	1.20 ^a	25
Glucose (mg/dl)	146.5	24	131.0 ^a	25	126.7 ^a	25
Potassium (mEq/L)	5.3	24	5.5	25	5.9 ^a	25
Calcium (mg/dl)	10.7	24	10.6	25	10.2 ^a	25
Sodium (mEq/L)	154.9	24	154.9	25	152.4 ^a	25
Bilirubin (mg/dl)	0.48	24	0.50	25	0.49	25
BUN (mg/dl)	17.8	24	17.4	25	20.3 ^a	25
Creatinine (mg/dl)	0.66	24	0.76 ^a	25	0.78 ^a	25
SGPT (IU/L)	52.3	24	43.5 ^a	25	37.6 ^a	25
SGOT (IU/L)	79.8	24	75.0	25	65.5 ^a	25
Alk. Phos. (IU/L)	11.4	24	11.1	25	9.9 ^a	25

^a Statistically different from controls, p < 0.01.

TABLE 25. HEMATOLOGY AND CLINICAL CHEMISTRY VALUES OF FEMALE RATS AFTER 90-DAY CONTINUOUS INHALATION EXPOSURE TO SHALE JP-5 VAPOR

	<u>Control</u>	<u>N</u>	<u>150 mg/m³</u>	<u>N</u>	<u>750 mg/m³</u>	<u>N</u>
RBC (10 ⁶)	8.8	25	8.6	25	8.5	25
WBC (10 ³)	5.7	25	5.2	25	4.7 ^a	25
HCT (%)	46.2	25	44.9	25	44.1 ^a	25
HGB (gm/dl)	14.8	25	14.6	25	14.2 ^a	25
MCV	52.9	25	52.5	25	52.2	25
MCH	17.0	25	17.1	25	16.9	25
MCHC	32.1	25	32.5	25	32.3	25
Total Pro. (gm/dl)	7.0	14	7.1	24	7.1	24
Albumin (gm/dl)	4.1	14	3.9	24	3.9	24
Globulin (gm/dl)	2.9	14	3.2 ^a	24	3.2 ^a	24
A/G Ratio	1.39	14	1.24 ^a	24	1.25 ^a	24
Glucose (mg/dl)	121.4	14	87.3 ^a	24	74.3 ^a	24
Potassium (mEq/L)	5.4	14	6.4 ^a	24	7.0 ^a	24
Calcium (mg/dl)	10.1	14	10.2	24	9.8	24
Sodium (mEq/L)	151.9	14	151.5	24	153.1	24
Bilirubin (mg/dl)	0.51	13	0.48	22	0.53	22
BUN (mg/dl)	22.2	12	15.1 ^a	22	18.9	21
Creatinine (mg/dl)	0.60	10	0.66	21	0.67	20
SGPT (IU/L)	52.3	14	43.5	24	46.0	24
SGOT (IU/L)	76.4	10	68.7	21	73.9	18
Alk. Phos. (IU/L)	6.8	13	7.1	24	6.6	23

^a Statistically different from controls, p < 0.01.

TABLE 26. THE EFFECT OF 90-DAY CONTINUOUS EXPOSURE
TO SHALE JP-5 ON ORGAN WEIGHTS OF MALE RATS

	<u>Control</u>	<u>N</u>	<u>150 mg/m³</u>	<u>N</u>	<u>750 mg/m³</u>
Body weight, gm	324.7 \pm 13.4	25	302.2 \pm 12.6 ^a	25	294.2 \pm 13.5 ^a
Liver weight., gms	8.77 \pm 0.53	24	7.38 \pm 0.55 ^a	25	8.69 \pm 0.52
Liver/100 g body wt.	2.70 \pm 0.10		2.44 \pm 0.12 ^a		2.95 \pm 0.13 ^a
Spleen wt., gms	0.58 \pm 0.06	23	0.57 \pm 0.06	25	0.58 \pm 0.07
Spleen/100 g body wt.	0.28 \pm 0.02		0.19 \pm 0.02		0.20 \pm 0.02 ^a
Kidney wt., gms	2.23 \pm 0.14	23	2.01 \pm 0.16 ^a	25	2.71 \pm 0.16 ^a
Kidney/100 g body wt.	0.69 \pm 0.03		0.66 \pm 0.04 ^b		0.92 \pm 0.04 ^a

^a Significantly different from control, p < 0.01.

^b Significantly different from control, p < 0.05.

TABLE 27. THE EFFECT OF 90-DAY CONTINUOUS EXPOSURE TO
SHALE JP-5 ON ORGAN WEIGHTS OF FEMALE RATS

	<u>Control</u>	<u>N</u>	<u>150 mg/m³</u>	<u>N</u>	<u>750 mg/m³</u>
Body weight, gm	173.8 \pm 7.1	25	168.3 \pm 7.4 ^a	25	157.6 \pm 13.2 ^a
Liver wt., gms	4.44 \pm 0.41	25	4.18 \pm 0.41 ^b	23	4.17 \pm 0.36 ^b
Liver/100 g body wt.	2.55 \pm 0.18		2.48 \pm 0.23		2.66 \pm 0.13 ^b
Spleen wt., gms	0.41 \pm 0.05	25	0.39 \pm 0.06	23	0.41 \pm 0.06
Spleen/100 g body wt.	0.23 \pm 0.03		0.23 \pm 0.04		0.26 \pm 0.03 ^a
Kidney wt., gms	1.29 \pm 0.08	25	1.20 \pm 0.11 ^a	23	1.23 \pm 0.09 ^b
Kidney/100 g body wt.	0.75 \pm 0.03		0.72 \pm 0.06 ^b		0.79 \pm 0.08 ^b

^a Significantly different from control, p < 0.01.

^b Significantly different from control, p < 0.05.

A SUBCHRONIC TOXICITY STUDY OF 90-DAY CONTINUOUS
INHALATION EXPOSURE TO PETROLEUM DIESEL FUEL MARINE

A 90-day continuous inhalation toxicity study of diesel fuel marine (DFM) vapor was conducted by the Toxic Hazards Research Unit during 1978. The DFM for the study was refined from petroleum oil. The U.S. Navy uses this type of diesel fuel as the fleet standard fuel for a large number of ships. To determine if replacement of petroleum DFM with shale derived fuels presented different health hazards, it was necessary to determine the toxicity of petroleum DFM for which information was lacking.

Beagle dogs, Fischer 344 rats, and C57B1/6 mice were continuously exposed to concentrations of 50 mg/m³ or 300 mg/m³ DFM vapor for 90 days in Thomas Dome inhalation chambers. Unexposed controls were held in laminar airflow rooms in separate facilities. At the conclusion of the exposure, all dogs and 1/3 of the rodents were sacrificed for tissue collection and histopathologic examination. The results of this examination were reported in a

previous report (MacEwen and Vernot, 1979). All rodents not sacrificed at the conclusion of the 90-day exposure were held for postexposure observation. An interim sacrifice of 1/2 of the remaining rodents occurred 19 months postexposure. Tissues were collected for histopathologic examination. Animals remaining from this interim sacrifice were held until the 24th month of the study, at which time all surviving animals were sacrificed for tissue examination in December 1979.

The body weight curves of male and female rats are shown in Figures 14 and 15. Both groups of petroleum DFM exposed male rats had depressed weight gains compared to unexposed control male rats after 2 weeks of exposure. The male rats exposed to 300 mg/m³ petroleum DFM vapor did not recover from the initial effect and the relative difference between this exposure group and the unexposed controls continued through the entire study. The male rats exposed to 50 mg/m³ petroleum DFM vapor returned to control body weight levels approximately 11 months postexposure. Body weights of the female rats exposed to 300 mg/m³ petroleum DFM vapor were statistically ($p < 0.05$) less than controls at the conclusion of the exposure phase of the study. This difference continued through the postexposure period. Female rats exposed to 50 mg/m³ DFM vapor did not show a difference in body weights from control rats until four months postexposure. At this point, the rate of weight gain became less and began to approximate the rate of the females exposed to 300 mg/m³.

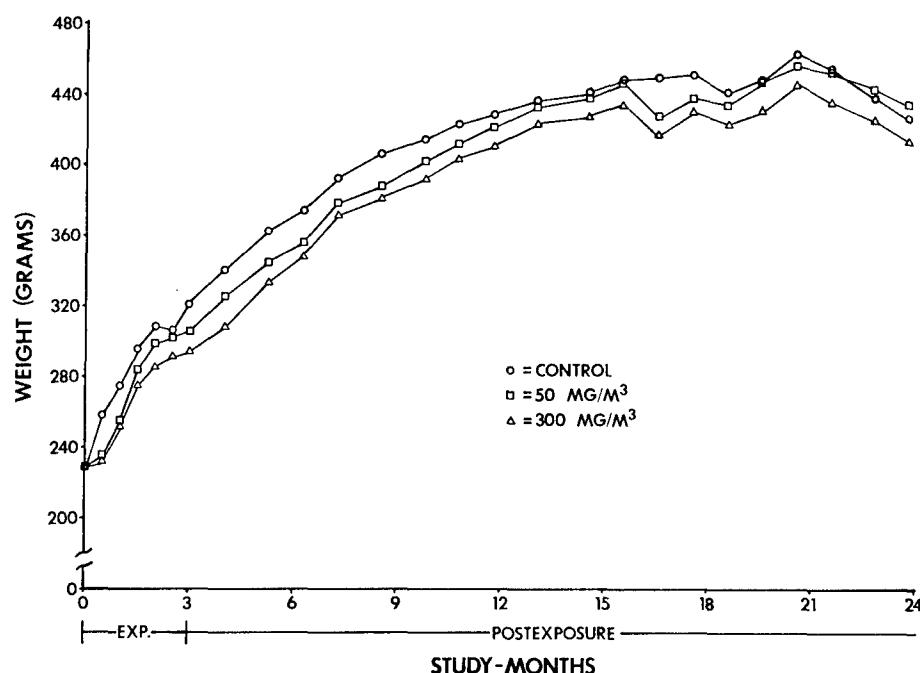


Figure 14. Effect of 90-day continuous exposure to petroleum DFM vapor on male rat body weight

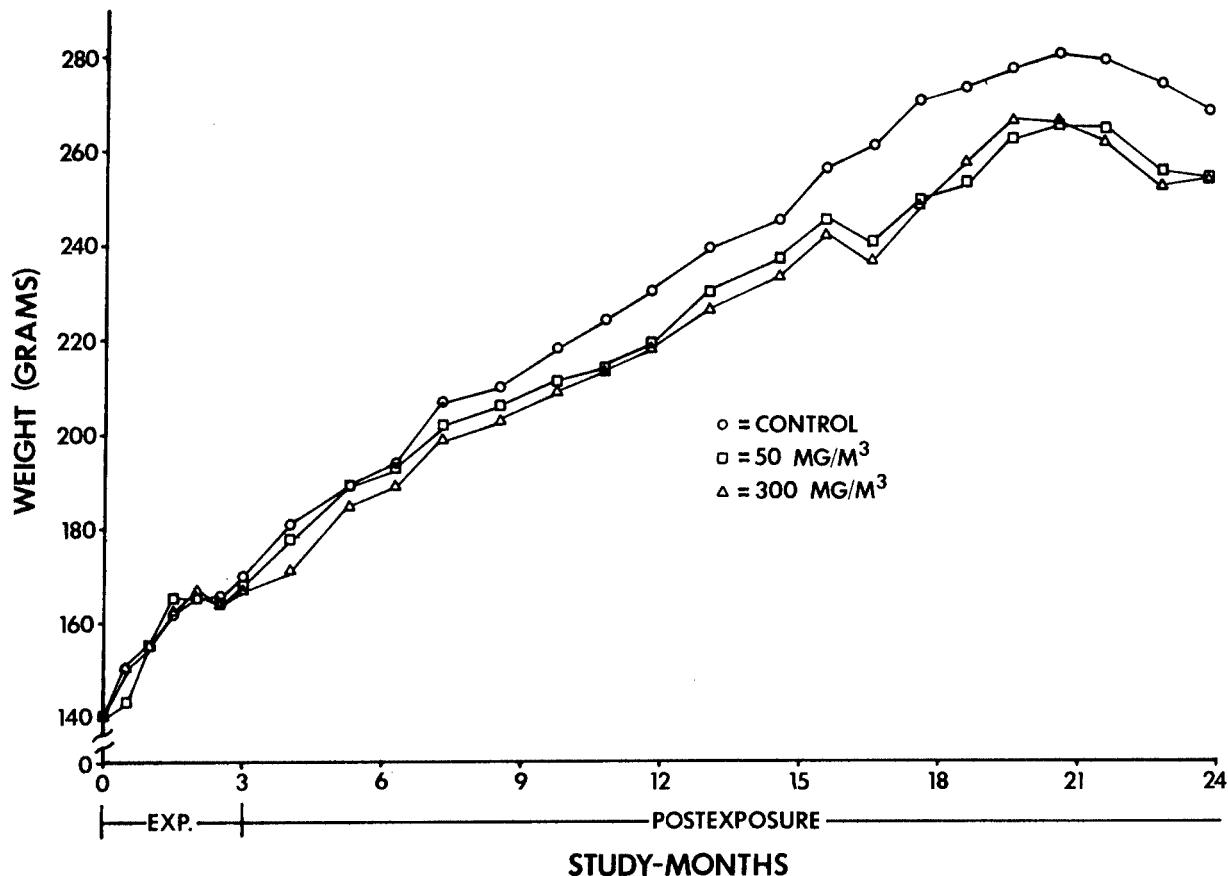


Figure 15. Effect of 90-day continuous exposure to petroleum DFM vapor on female rat body weight

Blood samples were obtained from the rats sacrificed at 19 months postexposure. The results are shown in Tables 28 and 29 for male and female rats, respectively. The creatinine value of the unexposed male control group is abnormally high. Two animals in this group had very high creatinine levels along with elevated BUN levels, possibly indicating kidney impairment. All other blood parameters for male and female rats are within normal limits.

Leukemic mononuclear cells were observed in three female control rats. These rats had white blood cell counts of over 40,000 per mm^3 . This type of leukemia was not observed in any of the petroleum DFM exposed animals. Goodman et al. (1979) have reported lymphomas and leukemias of all types to be very common in aging Fischer 344 rats.

TABLE 28. HEMATOLOGY AND CLINICAL CHEMISTRY VALUES OF
MALE RATS 19 MONTHS AFTER 90-DAY CONTINUOUS
INHALATION EXPOSURE TO PETROLEUM DFM VAPOR

	<u>Control</u>	<u>N</u>	<u>50 mg/m³</u>	<u>N</u>	<u>300 mg/m³</u>	<u>N</u>
RCB (10 ⁶)	7.9	20	8.9	15	8.9 ^a	21
WBC (10 ³)	6.2	20	6.8	15	6.1	21
HCT (%)	46.1	20	49.9	15	50.4	21
HGB (gm/dl)	15.1	20	16.2	15	16.5	21
MCV	58.2	20	56.0	15	56.4	21
MCH	18.9	20	18.3	15	18.4	21
MCHC	32.6	20	32.6	15	32.6	21
Total Pro. (gm/dl)	6.7	8	6.8	9	6.9	13
Albumin (gm/dl)	3.3	8	3.6	9	3.6	13
Globulin (gm/dl)	3.4	8	3.1	9	3.3	13
A/G ratio	0.98	8	1.16 ^a	9	1.08	13
Glucose (mg/dl)	106.8	8	148.7 ^a	9	150.1 ^a	13
Potassium (mEq/L)	5.2	8	5.1	9	5.0	13
Calcium (mg/dl)	10.2	8	9.3	9	10.6	13
Sodium (mEq/L)	150.9	8	153.8	9	152.0	13
Bilirubin (mg/dl)	0.63	8	0.57	9	0.53	13
BUN (mg/dl)	19.4	8	14.2	9	15.8	13
Creatinine (mg/dl)	0.93	8	0.67	9	0.75	13
SGPT (IU/L)	42.5	8	44.7	9	44.0	13
SGOT (IU/L)	82.8	8	94.4	9	73.4	13
Alk. Phos. (IU/L)	6.7	8	8.3	9	7.5	13

^a Statistically different from control, p < 0.01.

TABLE 29. HEMATOLOGY AND CLINICAL CHEMISTRY VALUES OF
FEMALE RATS 19 MONTHS AFTER 90-DAY CONTINUOUS
INHALATION EXPOSURE TO PETROLEUM DFM VAPOR

	<u>Control</u>	<u>N</u>	<u>50 mg/m³</u>	<u>N</u>	<u>300 mg/m³</u>	<u>N</u>
RCB (10 ⁶)	8.9	15	8.8	18	8.4	19
WBC (10 ³)	5.1	15	5.3	18	4.9	19
HCT (%)	46.2	15	45.3	18	42.9	19
HGB (gm/dl)	15.2	15	15.2	18	14.4	19
MCV	52.1	15	51.3	18	51.4	19
MCH	17.2	15	17.2	18	17.2	19
MCHC	32.9	15	33.5	18	33.5	19
Total Pro. (gm/dl)	7.6	4	7.9	11	7.8	18
Albumin (gm/dl)	4.0	4	4.3	11	4.2	18
Globulin (gm/dl)	3.6	4	3.6	11	3.6	18
A/G ratio	1.11	4	1.18	11	1.18	18
Glucose (mg/dl)	138.7	4	121.3	11	136.4	18
Potassium (mEq/L)	4.9	4	4.9	11	5.8 ^a	18
Calcium (mg/dl)	10.9	4	10.7	11	10.7	18
Sodium (mEq/L)	147.8	4	153.1 ^a	11	153.1 ^a	18
Bilirubin (mg/dl)	0.58	4	0.55	11	0.56	18
BUN (mg/dl)	15.2	4	15.7	11	15.9	18
Creatinine (mg/dl)	0.58	4	0.67	11	0.66	18
SGPT (IU/L)	54.5	4	54.7	11	51.1	18
SGOT (IU/L)	82.5	4	71.6	11	79.4	18
Alk. Phos. (IU/L)	7.7	4	6.0	11	8.6	18

^a Statistically different from control, p < 0.01.

The organ weights of rats sacrificed at 19 months postexposure are shown in Tables 30 and 31 for male and female rats, respectively. The body weights of rats in the exposure groups were generally less than unexposed controls. These decreases were reflected in the absolute organ weights, particularly the liver and kidney weights of the rats exposed to 300 mg/m³ petroleum DFM. The mean spleen weights and other organ weights of female rats in the unexposed control group were extremely high as a result of three rats in the group that had very heavy spleens. Two of the spleens weighed in excess of 5 grams. The third weighed in excess of 20 grams. These three rats are the same rats mentioned previously in this report that had leukemic mononuclear cells.

TABLE 30. MEAN ORGAN WEIGHTS OF MALE RATS 19 MONTHS AFTER 90-DAY CONTINUOUS EXPOSURE TO PETROLEUM DFM VAPORS

	<u>Control</u>	<u>N</u>	<u>50 mg/m³</u>	<u>N</u>	<u>300 mg/m³</u>	<u>N</u>
Body weight, gm	419.9 \pm 39.9	20	425.7 \pm 35.6	15	403.9 \pm 28.1	21
Liver wt., gms	12.46 \pm 1.95	20	11.84 \pm 1.19	15	11.31 \pm 1.38 ^b	20
Liver/100 g body wt.	3.01 \pm 0.70		2.78 \pm 0.22		2.82 \pm 0.39	
Spleen wt., gms	0.99 \pm 0.77	20	0.94 \pm 0.49	15	0.99 \pm 0.66	21
Spleen/100 g body wt.	0.24 \pm 0.19		0.22 \pm 0.12		0.25 \pm 0.17	
Kidney wt., gms	2.97 \pm 0.29	20	2.87 \pm 0.19	15	2.78 \pm 0.27	21
Kidney/100 g body wt.	0.72 \pm 0.14		0.68 \pm 0.05		0.69 \pm 0.07	

^a Mean \pm S.D.

^b Significant test vs. control, p < 0.05.

TABLE 31. MEAN ORGAN WEIGHTS OF FEMALE RATS 19 MONTHS AFTER 90-DAY CONTINUOUS EXPOSURE TO PETROLEUM DFM VAPORS

	<u>Control</u>	<u>N</u>	<u>50 mg/m³</u>	<u>N</u>	<u>300 mg/m³</u>	<u>N</u>
Body weight, gm	259.1 \pm 22.9	18	247.2 \pm 21.0	18	245.9 \pm 24.4	19
Liver wt., gms	7.64 \pm 0.95	18	6.98 \pm 0.75 ^b	18	6.76 \pm 0.82 ^a	19
Liver/100 g body wt.	2.97 \pm 0.48		2.82 \pm 0.19		2.75 \pm 0.19	
Spleen wt., gms	2.12 \pm 4.7	18	0.45 \pm 0.10	18	0.65 \pm 0.67	19
Spleen/100 g body wt.	0.88 \pm 1.93		0.18 \pm 0.05		0.26 \pm 0.25	
Kidney wt., gms	2.17 \pm 0.56	18	1.95 \pm 0.23	18	1.83 \pm 0.19 ^b	19
Kidney/100 g body wt.	0.84 \pm 0.24		0.79 \pm 0.06		0.75 \pm 0.10	

^a Significant test vs. control, p < 0.01.

^b Significant test vs. control, p < 0.05.

Histopathologic examination of the tissues obtained from the dogs sacrificed at the conclusion of the 90-day exposure revealed an increased frequency of cytoplasmic vacuolization of hepatocytes. This lesion is interpreted as a mild manifestation of cytotoxicity or a predisposition for hepatocytes to accumulate excessive glycogen. All other lesions noted in the dogs were interpreted as incidental findings.

Histopathologic examination of the tissues from the interim and final sacrifices was not complete for this report.

A SUBCHRONIC TOXICITY STUDY OF A 90-DAY CONTINUOUS INHALATION EXPOSURE TO SHALE DIESEL FUEL MARINE

This study was a continuation of a series of studies conducted by the Toxic Hazards Research Unit for the U.S. Navy to evaluate the inhalation toxicity of hydrocarbon fuels derived from petroleum and oil shale sources. The material used in the study was shale diesel fuel marine (DFM) derived from Colorado oil shale.

In the previous inhalation study, male rats exposed to petroleum diesel fuel vapors at concentrations of 50 mg/m³ or 300 mg/m³ had significantly retarded body weight gains compared to unexposed control male rats. Renal tissue lesions consisting of hyaline droplet formation, nephropathy localized at the cortico-medullary junction, and interstitial nephritis were found in the male rats exposed to petroleum DFM vapors. Unexposed control male rats and all female rats were free of these lesions. Various blood parameters measured in beagle dogs failed to reveal any abnormalities attributable to petroleum DFM exposure.

The present study involved the exposure of groups of 3 male and 3 female beagle dogs, 75 male and 75 female Fischer 344 rats, and 150 female C57B1/6 mice. A similar number of unexposed control animals were housed in laminar airflow facilities in a separate building. Two shale DFM exposure concentrations were used, 50 mg/m³ and 300 mg/m³. These concentrations were the same as those used in the study of petroleum DFM.

The generation and analysis system used for shale DFM vapors resembled that used for the generation of most hydrocarbon fuels and is described in the section on petroleum JP-4 and shown in Figures 3 and 4 of this report. It was necessary, however, to add a third vapor generating tower in order to produce enough vapor from the shale DFM which was less volatile than previous hydrocarbon fuels studied. Two of the evaporator tower outputs were mixed and split between the two exposure chambers. The output of the third tower went directly into the higher concentration chamber. Operating conditions of the evaporator towers were identically maintained to assure similarity in the shale DFM vapor constituents within the chamber.

The experimental protocol followed was similar to that used in the study of petroleum DFM. Exposures were conducted on a continuous basis for a 90-day period. Upon termination of the 90-day exposure, all dogs and one third of the rodents from each exposure group and controls were sacrificed for gross and histopathologic examination to detect any pathological lesions caused by exposure to shale DFM.

Prior to the initiation of the exposures, drums of shale DFM were analyzed with a gas chromatograph to insure that an adequate supply of shale DFM was available for a 90-day continuous exposure. The measured exposure means and standard deviations for the entire study were $50.3 \pm 0.5 \text{ mg/m}^3$ and $300.2 \pm 3.7 \text{ mg/m}^3$.

Exposure to shale DFM had no effect on the body weight gains in beagle dogs. Transient changes in various blood parameters were noted in dogs exposed to shale DFM vapors for 90 days. However, all values were within normal limits for the species with the exception of one male dog in the 50 mg/m^3 shale DFM group. The SGPT level in this dog increased to twice the normal value after one week of exposure. By the fourth week of exposure, the SGPT level had returned to normal. The increase was probably not exposure-related. Red blood cell osmotic fragility was not affected by exposure to shale DFM vapors (Table 32).

TABLE 32. EFFECT OF 90-DAY CONTINUOUS EXPOSURE TO SHALE DFM VAPORS ON RED BLOOD CELL FRAGILITY^a OF BEAGLE DOGS

Exposure Weeks	Control	50 mg/m^3	300 mg/m^3	Saline Conc.
0	2.9 ± 0.9	3.5 ± 1.2	2.9 ± 1.9	0.50%
4	2.9 ± 0.9	3.9 ± 2.8	2.4 ± 1.6	
8	6.7 ± 1.4	8.9 ± 3.3	6.8 ± 3.5	
12	5.4 ± 2.4	5.3 ± 1.0	6.4 ± 3.5	
0	5.3 ± 1.9	8.2 ± 3.7	5.6 ± 2.9	0.475%
4	6.0 ± 2.3	8.1 ± 3.4	4.9 ± 4.3	
8	12.6 ± 1.2	18.2 ± 3.4	14.5 ± 8.5	
12	12.5 ± 4.3	12.4 ± 2.9	12.7 ± 8.3	
0	12.9 ± 4.7	23.9 ± 10.9	17.6 ± 14.7	0.45%
4	17.5 ± 8.4	25.3 ± 11.1	16.2 ± 13.4	
8	34.2 ± 4.9	43.3 ± 12.3	36.5 ± 18.1	
12	28.5 ± 6.5	34.2 ± 6.9	29.7 ± 17.5	
0	35.5 ± 10.2	40.6 ± 17.6	35.8 ± 23.7	0.425%
4	33.7 ± 14.6	45.2 ± 13.9	28.6 ± 14.8	
8	62.6 ± 9.9	72.7 ± 5.3	61.4 ± 16.9	
12	54.4 ± 11.8	59.8 ± 6.0	54.7 ± 18.2	
0	58.9 ± 9.7	70.9 ± 18.0	61.3 ± 20.5	0.40%
4	66.1 ± 14.9	78.3 ± 11.9	57.3 ± 15.7	
8	83.9 ± 6.6	89.7 ± 7.9	82.5 ± 11.2	
12	81.4 ± 11.3	84.6 ± 4.8	77.4 ± 13.9	
0	80.1 ± 7.6	85.4 ± 7.2	81.6 ± 11.1	0.375%
4	87.8 ± 6.7	93.4 ± 4.2	82.7 ± 8.2	
8	95.7 ± 3.1	94.9 ± 6.2	91.9 ± 5.6	
12	93.7 ± 4.9	96.4 ± 2.6	91.2 ± 6.9	

^a % hemolysis, mean \pm S.D., N = 6 dogs/group (3 male, 3 female)

Organ weights obtained from dogs at the conclusion of the 90-day exposure are shown in Table 33.

TABLE 33. EFFECT OF 90-DAY CONTINUOUS EXPOSURE TO SHALE DFM VAPORS ON ORGAN WEIGHTS^a OF BEAGLE DOGS

	<u>Control</u>	<u>50 mg/m³</u>	<u>300 mg/m³</u>
Body weight, kg	10.4 \pm 0.6	11.9 \pm 1.5 ^b	12.0 \pm 1.4 ^b
Liver wt., gms	314.0 \pm 3.6	327.1 \pm 43.9	384.7 \pm 49.8 ^b
Liver/100 g body wt.	3.0 \pm 0.3	2.8 \pm 0.3	3.2 \pm 0.6
Spleen wt., gms	42.5 \pm 17.4	93.5 \pm 35.8 ^b	86.3 \pm 36.9 ^b
Spleen/100 g body wt.	0.4 \pm 0.2	0.8 \pm 0.4 ^b	0.7 \pm 0.3
Kidney wt., gms	4.4 \pm 0.2	5.1 \pm 0.8	5.1 \pm 0.9
Kidney/100 g body wt.	0.04 \pm 0.003	0.04 \pm 0.01	0.04 \pm 0.01

^a Mean \pm S.D., N = 6

^b Statistically different from controls (p < 0.05).

Both groups of dogs exposed to shale DFM vapors weighed more than control dogs at the conclusion of the exposure. Increases in absolute liver and spleen weights are also noted. However, when the organ/body ratios are compared, only the spleens of the dogs exposed to 50 mg/m³ shale DFM vapors show a significant (p < 0.05) increase over unexposed control dog values. The spleen/body weight ratio of the dogs exposed to 300 mg/m³ was also greater than the control value; however, the increase was not statistically significant at p < 0.05.

The body weights of male and female rats are shown in Figures 16 and 17, respectively. Exposure to 300 mg/m³ shale DFM vapors for 90 days retarded the body weight gain of male rats when compared to unexposed control male rats. This effect was not evident in the male rats exposed to 50 mg/m³ shale DFM vapors. A similar effect was seen in the female rats when compared to unexposed controls. Exposure to the lower concentration of shale DFM had no effect on female rat body weight.

Rat hematological and clinical chemistry values obtained at the conclusion of the 90-day exposure are shown in Tables 34 and 35 for male and female rats, respectively. A number of differences between the test groups and control group are indicated. These differences are probably not biologically significant since all of the values are within normal limits for this species.

Male rat organ weights obtained at the conclusion of the exposures are shown in Table 36. Kidney weights in male rats exposed to 300 mg/m³ shale DFM vapor were increased over those of unexposed controls and, along with the increased BUN and creatinine levels in these rats, may indicate renal injury as was seen in male rats exposed to other hydrocarbon fuels.

Female rat organ weights are shown in Table 37. Increased liver weight was evident in the female rats exposed to 300 mg/m³ shale DFM vapor when compared to unexposed control values. As with the male rats, the organ weights of the female rats exposed to 50 mg/m³ shale DFM vapor were generally less than control values. In the case of kidney weights, the difference in values was statistically significant.

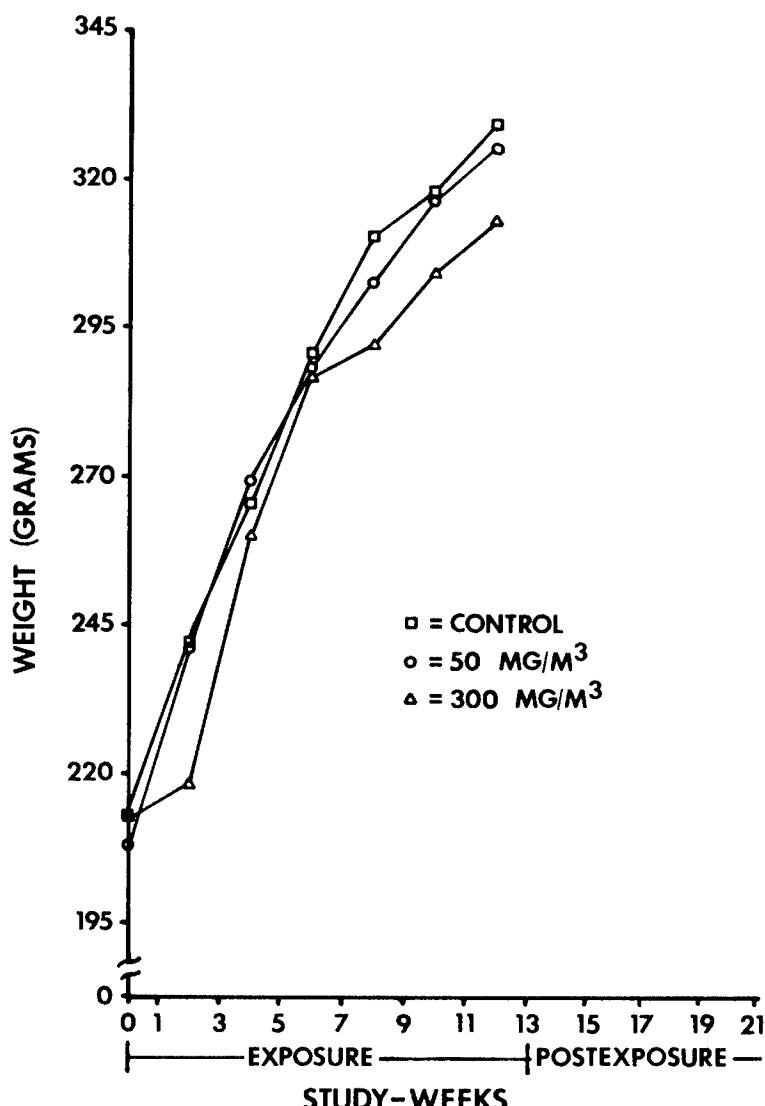


Figure 16. Effect of 90-day continuous inhalation exposure to shale DFM vapor on male rat body weight.

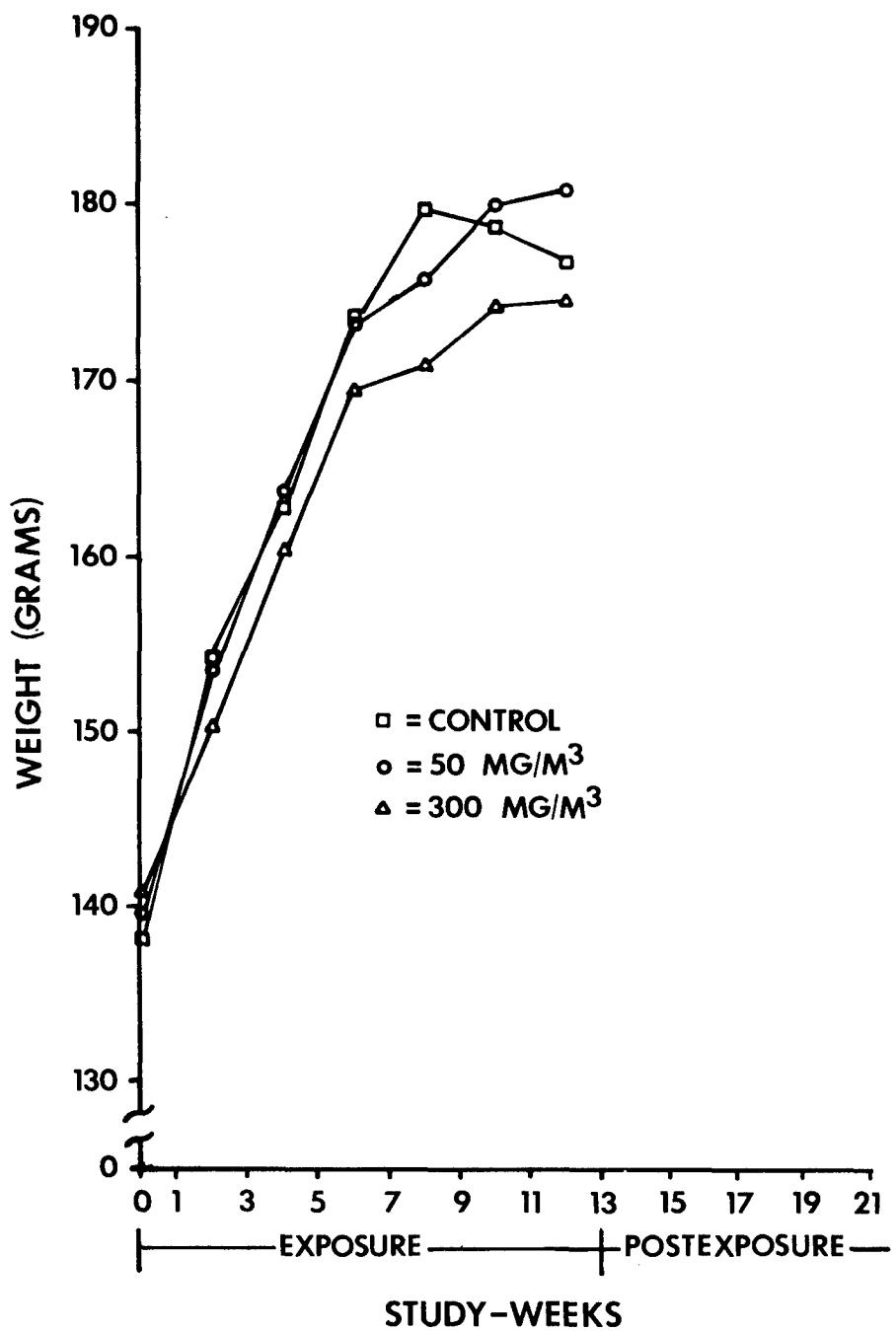


Figure 17. Effect of 90-day continuous inhalation exposure to shale DFM vapor on female rat body weight.

TABLE 34. HEMATOLOGY AND CLINICAL CHEMISTRY VALUES OF
MALE RATS AFTER 90-DAY CONTINUOUS INHALATION
EXPOSURE TO SHALE DFM VAPOR

	<u>Control</u> N=25	<u>50 mg/m³</u> N=24	<u>300 mg/m³</u> N=25
RBC (10 ⁶)	8.9	8.5 ^a	8.3 ^a
WBC (10 ³)	5.6	5.9	5.9
HCT (%)	45.4	44.5	43.1 ^a
HGB (gm/dl)	15.1	14.9	14.8
MCV	50.9	52.5	52.0
MCH	16.9	17.7	17.9 ^a
MCHC	33.2	33.6	34.4
Total Pro. (gm/dl)	7.2	7.2	7.3
Albumin (gm/dl)	4.2	4.0 ^a	4.1 ^a
Globulin (gm/dl)	2.9	3.1 ^a	3.2 ^a
A/G ratio	1.5	1.3 ^a	1.3 ^a
Glucose (mg/dl)	143.0	140.5	125.8 ^a
Potassium (mEq/L)	5.9	5.7	5.6
Calcium (mg/dl)	10.8	10.7	10.8
Sodium (mEq/L)	149.7	149.3	150.4
Bilirubin (mg/dl)	0.49 ^b	0.47	0.46
BUN (mg/dl)	16.5 ^b	16.4	17.9 ^a
Creatinine (mg/dl)	0.36 ^b	0.47 ^a	0.55 ^a
SGPT (IU/L)	54.7	45.0	44.2
SGOT (IU/L)	85.7	83.6	80.5
Alk. Phos. (IU/L)	10.1	10.0	9.6

^a Significantly different from control, p < 0.01.

^b N = 23.

The exposure phase of this study was completed in March 1980. Histopathologic examination of the tissues obtained from the dogs, rats, and mice sacrificed at that time was not available for this report. Therefore, it is not clear if the differences noted in rat body weights, blood values, and organ weights are actual indications of tissue injury.

The results obtained through the completion of the 90-day exposure to shale DFM vapors are similar to the results of the 90-day exposure to petroleum DFM (MacEwen and Vernot, 1978). Information will be presented in future annual reports concerning the histological examination of tissues as well as the progress of postexposure observation.

TABLE 35. HEMATOLOGY AND CLINICAL CHEMISTRY VALUES OF FEMALE RATS AFTER 90-DAY CONTINUOUS INHALATION EXPOSURE TO SHALE DFM VAPOR

	<u>Control</u>	<u>N</u>	<u>50 mg/m³</u>	<u>N</u>	<u>300 mg/m³</u>	<u>N</u>
RCB (10 ⁶)	7.8	25	6.0	25	7.9	25
WBC (10 ³)	4.2	25	4.7	25	3.9	25
HCT (%)	41.9	25	42.0	25	41.3	25
HGB (gm/dl)	14.8	25	14.9	25	14.6	25
MCV	53.8	25	52.6	25	52.6	25
MCH	18.9	25	18.6	25	18.6	25
MCHC	35.3	25	35.4	25	35.4	25
Total Pro. (gm/dl)	7.1	22	7.2	24	7.4 ^a	23
Albumin (gm/dl)	4.3	22	4.2	24	4.2	22
Globulin (gm/dl)	2.9	22	3.0	24	3.2 ^a	22
A/G ratio	1.5	22	1.4 ^a	24	1.3 ^a	22
Glucose (mg/dl)	116.3	22	101.5 ^a	24	95.4 ^a	23
Potassium (mEq/L)	4.7	22	4.3	24	4.8	24
Calcium (mg/dl)	10.6	22	10.5	24	10.4	23
Sodium (mEq/L)	150.9	22	147.4 ^a	24	148.5 ^a	24
Bilirubin (mg/dl)	0.44	19	0.39 ^a	23	0.40	21
BUN (mg/dl)	16.3	18	17.4	21	17.4	19
Creatinine (mg/dl)	0.38	18	0.46	21	0.44	19
SGPT (IU/L)	75.1	22	49.3 ^a	24	44.9 ^a	23
SGOT (IU/L)	109.2	22	97.4	24	86.7 ^a	23
Alk. Phos. (IU/L)	4.4	20	4.9	24	5.9 ^a	21

^a Significantly different from control, p < 0.01.

TABLE 36. THE EFFECT OF 90-DAY CONTINUOUS EXPOSURE TO SHALE DFM VAPOR ON ORGAN WEIGHTS OF MALE RATS

	<u>Control</u>	<u>50 mg/m³</u>	<u>300 mg/m³</u>
	<u>N = 22</u>	<u>N = 21</u>	<u>N = 25</u>
Body weight, gm	310.4 \pm 19.2	310.8 \pm 16.4	296.0 \pm 26.9 ^a
Liver wt., gms	8.1 \pm 0.7	7.8 \pm 16.4	8.0 \pm 0.8
Liver/100 g body wt.	2.60 \pm 0.16	2.49 \pm 0.11 ^a	2.72 \pm 0.13 ^b
Spleen wt., gms	0.55 \pm 0.05	0.57 \pm 0.07	0.55 \pm 0.06
Spleen/100 g body wt.	0.18 \pm 0.02	0.18 \pm 0.02	0.19 \pm 0.01
Kidney wt., gms	2.07 \pm 0.16	1.99 \pm 0.15 ^b	2.24 \pm 0.22 ^b
Kidney/100 g body wt.	0.67 \pm 0.03	0.64 \pm 0.03 ^b	0.76 \pm 0.04 ^b

^a Statistically different from control, p < 0.05.

^b Statistically different from control, p < 0.01.

TABLE 37. EFFECT OF 90-DAY CONTINUOUS EXPOSURE TO SHALE DFM VAPOR ON ORGAN WEIGHTS OF FEMALE RATS

	<u>Control</u>	<u>50 mg/m³</u>	<u>300 mg/m³</u>
	N = 25	N = 25	N = 25
Body weight, gms	168.5 \pm 9.7	168.5 \pm 11.5	165.4 \pm 7.7
Liver wt., gms	4.24 \pm 0.35	4.11 \pm 0.48	4.43 \pm 0.42
Liver/100 g body wt.	2.51 \pm 0.13	2.44 \pm 0.18	2.68 \pm 0.21 ^a
Spleen wt., gms	0.37 \pm 0.03	0.37 \pm 0.04	0.37 \pm 0.05
Spleen/100 g body wt.	0.22 \pm 0.17	0.22 \pm 0.02	0.23 \pm 0.02
Kidney wt., gms	1.19 \pm 0.08	1.12 \pm 0.08 ^a	1.16 \pm 0.08
Kidney/100 g body wt.	0.71 \pm 0.05	0.66 \pm 0.03 ^a	0.70 \pm 0.04

^a Statistically different from control, p < 0.01.

A SUBCHRONIC TOXICITY STUDY OF 90-DAY CONTINUOUS INHALATION EXPOSURE TO DECALIN VAPOR

Decalin (decahydronaphthalene) is an alicyclic hydrocarbon commonly used as a solvent for oil, fats, and resins and has been substituted for turpentine in oil paints. Another important use of decalin is as a solvent and stabilizer for shoe creams and floor waxes, in which the mild terpene-like odor can be easily hidden. It also has desirable characteristics for use as a high density fuel and as such could present new health hazards to military personnel.

The Toxic Hazards Research Unit previously conducted a one-month intermittent inhalation exposure study of rats, mice, and guinea pigs to decalin vapor (MacEwen and Vernot, 1978). The incidence of pathologic lesions noted in animals exposed to decalin in that study revealed a need for more information. The present subchronic study was designed to determine the toxic effects of a 90-day continuous exposure of test animals to decalin vapor.

Dogs, rats, and mice were continuously exposed for 90 days to 5 or 50 ppm decalin in Thomas Dome inhalation chambers. Control animals were housed in laminar air flow facilities. Upon termination of the exposures, all of the dogs and one-third of the rodents were sacrificed for tissue collection and examination. One-half of the remaining rats were sacrificed after 19 months of postexposure observation for tissue collection and examination. Animals remaining from this interim sacrifice were held until the 24th month of the study at which time a final sacrifice occurred.

A more detailed discussion of the experimental design can be found in the previous THRU annual report (MacEwen and Vernot, 1979). This report presents results which have become available since the conclusion of the exposure.

Exposure to decalin vapor affected the weight of the male Fischer 344 rats as shown in Figure 18. Both groups of decalin exposed animals weighed statistically ($p < 0.05$) less than unexposed control animals through the exposure phase and through most of the postexposure phase. A dose-related effect is suggested by the weight curve. However, this was not a consistent statistical finding. Female rat body weights are shown in Figure 19 and indicate that exposure to decalin vapor had little effect.

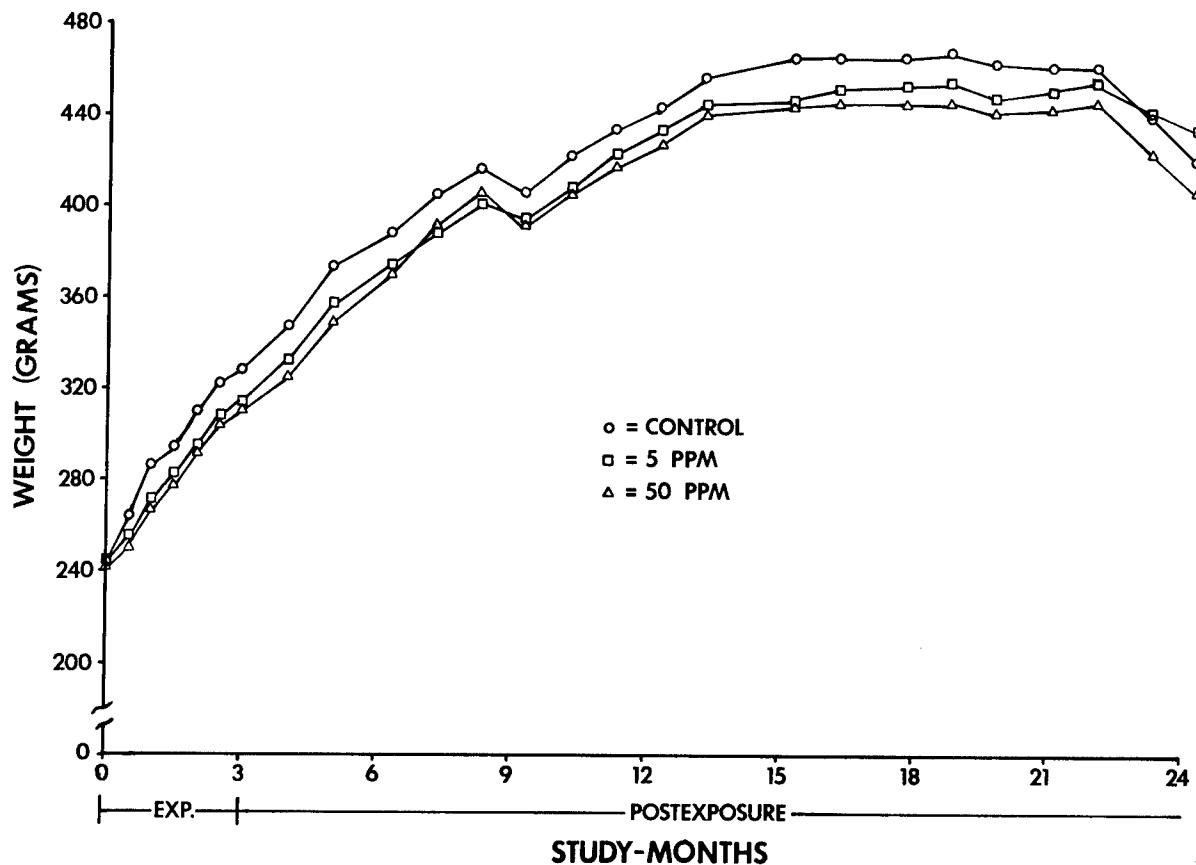


Figure 18. Effect of 90-day continuous inhalation exposure to decalin vapor on male rat body weight.

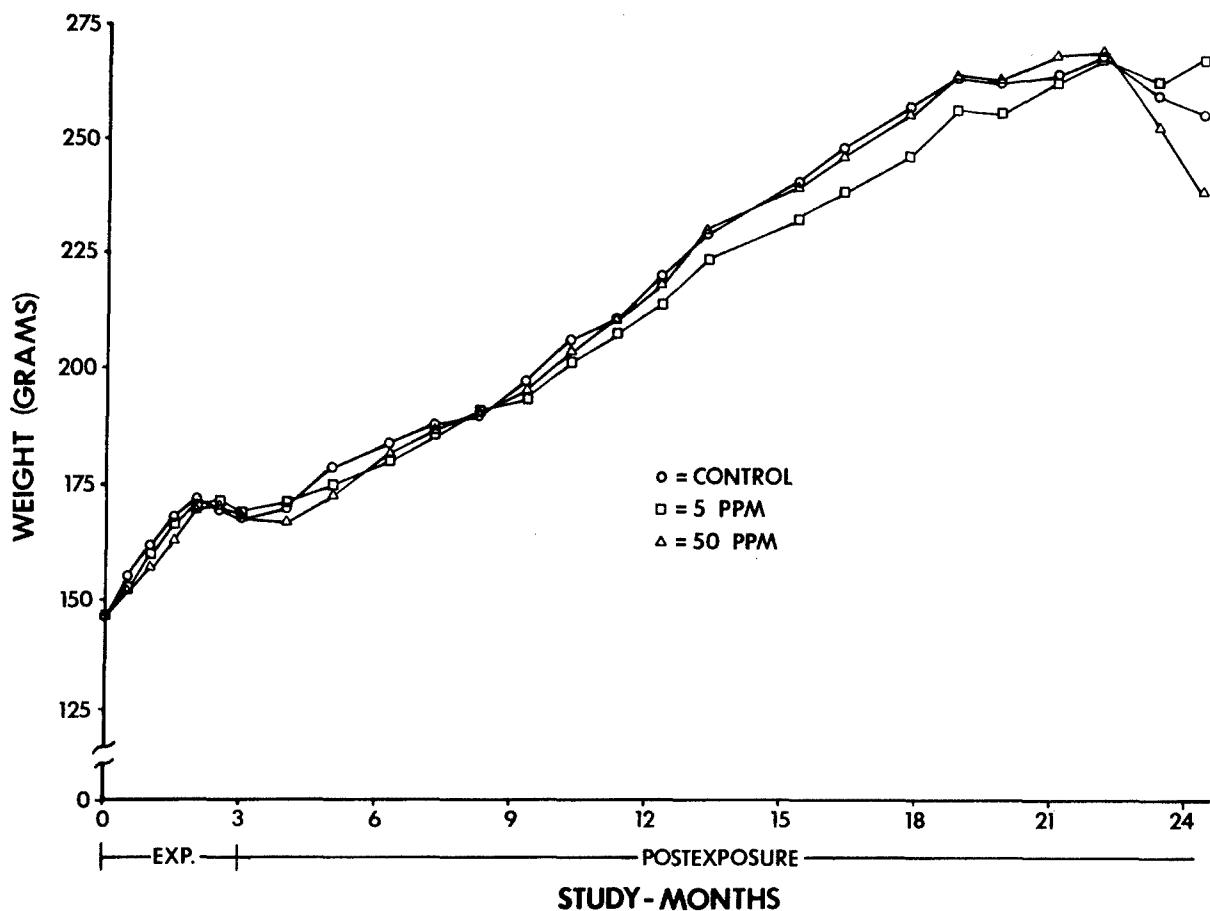


Figure 19. Effect of 90-day continuous inhalation exposure to decalin vapor on female rat body weight.

Blood samples were obtained from the rats necropsied at 19-months postexposure. The mean values are shown in Tables 38 and 39 for males and females, respectively. A few of the values obtained are statistically different from respective unexposed control rats. However, the differences probably have no biological significance. Leukemic mononuclear cells were observed in one male rat exposed to 5.0 ppm decalin and in two female rats exposed to 5.0 ppm decalin. Lymphomas and leukemias of all types are among common tumors found in aging Fischer 344 rats (Goodman et al., 1979). As reported previously, the blood values obtained from the rats necropsied at the conclusion of the 90-day exposure were rejected because almost all were hemolyzed samples. Therefore, a comparison between those values and the 19-months post-exposure values is not possible.

TABLE 38. HEMATOLOGY AND CLINICAL CHEMISTRY VALUES OF MALE RATS OBTAINED 19-MONTHS AFTER 90-DAY CONTINUOUS EXPOSURE TO DECALIN VAPOR

	Control	N	5 ppm	N	50 ppm	N
RBC (10^6)	9.5	16	8.8	17	8.8	20
WBC (10^3)	5.6	16	5.4	17	6.2	20
HCT (%)	54.3	16	50.0	17	50.2	20
HGB (gm/dl)	18.4	16	17.0	17	17.2	20
MCV	57.3	16	56.6	17	57.1	20
MCH	19.4	16	19.3	17	19.6	20
MCHC	33.8	16	34.1	17	34.3	20
Total Pro. (gm/dl)	7.09	9	7.1	15	7.0	17
Albumin (gm/dl)	4.0	9	3.9	15	7.0	17
Globulin (gm/dl)	4.0	9	3.9	15	3.9	17
A/G Ratio	1.33	9	1.27	15	1.27	17
Glucose (mg/dl)	171.9	9	144.1 ^a	15	149.0 ^a	17
Potassium (mEq/L)	5.0	9	4.8	15	4.8	17
Calcium (mg/dl)	11.7	9	11.4	15	11.3	17
Sodium (mEq/L)	150.0	9	149.9	15	149.2	17
Bilirubin (mg/dl)	0.63	8	0.60	15	0.60	16
BUN (mg/dl)	17.9	8	17.3	15	16.9	16
Creatinine (mg/dl)	0.45	9	0.43	15	0.52	15
SGPT (IU/L)	54.0	9	49.5	15	45.6	17
SGOT (IU/L)	102.0	9	90.3	15	83.9	17
Alk. Phos. (IU/L)	19.8	8	9.1	15	7.8	17

^a Significantly different from control values, $p < 0.05$.

TABLE 39. HEMATOLOGY AND CLINICAL CHEMISTRY VALUES OF FEMALE RATS OBTAINED 19-MONTHS AFTER 90-DAY CONTINUOUS EXPOSURE TO DECALIN VAPOR

	Control	N	5 ppm	N	50 ppm	N
RBC (10^6)	7.9	17	7.9	19	8.1	18
WBC (10^3)	5.7	17	5.0	19	4.2	18
HCT (%)	42.9	17	42.8	19	43.1	18
HGB (gm/dl)	13.9	17	14.4	19	14.3	18
MCV	54.3	17	43.7	19	53.5	18
MCH	17.6	17	18.0	19	17.8	18
MCHC	32.5	17	33.5 ^a	19	33.3	18
Total Pro. (gm/dl)	7.4	16	7.3	15	7.5	18
Albumin (gm/dl)	4.4	16	4.3	15	4.4	18
Globulin (gm/dl)	3.1	16	3.0	15	3.1	18
A/G Ratio	1.43	16	1.42	15	1.42	18
Glucose (mg/dl)	164.2	16	156.5	15	143.3 ^b	18
Potassium (mEq/L)	4.9	16	5.2	15	5.3	18
Calcium (mg/dl)	11.2	16	10.9	15	11.0	18
Sodium (mEq/L)	141.6	16	139.8	15	143.8 ^a	18
Bilirubin (mg/dl)	0.56	16	0.53	15	0.53	18
BUN (mg/dl)	17.1	16	16.9	15	15.6	18
Creatinine (mg/dl)	0.53	16	0.32	15	0.28	18
SGPT (IU/L)	53.5	16	0.32	15	0.28	18
SGOT (IU/L)	86.0	16	80.9	15	73.0 ^a	18
Alk. Phos. (IU/L)	15.9	16	14.2	15	6.7	18

^a Statistically different from control values, $p < 0.05$.

^b Statistically different from control values, $p < 0.01$.

Organ weights obtained from the rats sacrificed at 19 months postexposure are shown in Tables 40 and 41 for males and females, respectively.

TABLE 40. ORGAN WEIGHTS OF MALE RATS 19 MONTHS AFTER 90-DAY CONTINUOUS EXPOSURE TO DECALIN

	<u>Control</u>	<u>5 ppm</u>	<u>50 ppm</u>
Body weight, gm	431.8 \pm 27.4	421.1 \pm 21.7	409.7 \pm 26.4 ^a
Liver wt., gms	12.27 \pm 1.08	11.66 \pm 1.54	11.95 \pm 1.09
Liver/100 g body wt.	2.85 \pm 0.27	2.78 \pm 0.42	2.93 \pm 0.33
Spleen wt., gms	1.19 \pm 0.54	2.03 \pm 4.54	1.41 \pm 1.17
Spleen/100 g body wt.	0.28 \pm 0.13	0.50 \pm 1.18	0.35 \pm 0.29
Kidney wt., gms	2.98 \pm 0.32	2.93 \pm 0.18	3.01 \pm 0.18
Kidney/100 g body wt.	0.69 \pm 0.08	0.69 \pm 0.06	0.74 \pm 0.04

^a Statistically different from controls at $p < 0.05$.

TABLE 41. ORGAN WEIGHTS OF FEMALE RATS 19 MONTHS AFTER 90-DAY CONTINUOUS EXPOSURE TO DECALIN

	<u>Control</u>	<u>5 ppm</u>	<u>50 ppm</u>
Body weight, gms	253.2 \pm 33.2	249.0 \pm 27.5	249.7 \pm 22.7
Liver wt., gms	6.94 \pm 1.78	6.46 \pm 1.00	6.57 \pm 0.82
Liver/100 g body wt.	2.74 \pm 0.47	2.66 \pm 0.50	2.65 \pm 0.28
Spleen wt., gms	0.67 \pm 0.42	1.03 \pm 1.93	0.54 \pm 0.32
Spleen/100 g body wt.	0.27 \pm 0.18	0.45 \pm 0.92	0.22 \pm 0.13
Kidney wt., gms	1.79 \pm 0.17	1.72 \pm 0.13	1.82 \pm 0.16
Kidney/100 g body wt.	0.72 \pm 0.07	0.69 \pm 0.09	0.74 \pm 0.10

No statistical differences between decalin exposed and unexposed control rat organ weights was seen. One male rat in the 5.0 ppm decalin exposure group had a spleen weight of over 20 grams. This animal was the same animal that had leukemic mononuclear cells. The two female rats with leukemic mononuclear cells also had enlarged spleens; however, this condition was not as great as in the male rat. These enlarged organs are responsible for the wide standard deviations in spleen weights of the male and female rats in the 5.0 ppm exposure groups.

Clearly dose-related lesions were not observed either grossly or microscopically in dogs exposed to decalin vapors. Of moderate significance, however, was the finding of pulmonary inflammatory lesions in both control and exposed groups. These inflammatory changes ranged from mild, focal, chronic lesions in some dogs to diffuse, chronic active bronchopneumonia in others. In most cases, inflammation was attended by abundant eosinophil infiltrates.

Lesions in rats necropsied at the conclusion of the 90-day exposure that were considered to be exposure-related occurred only in male rats and were restricted to the kidneys where 100% of the 5 ppm decalin exposure group and 96% of the 50 ppm decalin exposure group exhibited changes compatible with toxic tubular nephrosis. The lesions were dose-related in severity. Lesions of this type were not observed in female rats or in the control animals. With light microscopy, the most striking lesions consisted of mild to moderate focal necrosis of tubular epithelial cells at the level of the corticomedullary junction with mild, cystic tubular dilatation and intraluminal casts of granular, amorphous cellular debris. The tubule retained a lining of flattened, stretched-out epithelium where persisting cells attempted to resurface the denuded tubular basement membrane. These tubular changes were usually accompanied by the presence of moderately abundant cytoplasmic hyalin droplets in the proximal tubular epithelial cells. Hyalin droplets are regarded as microscopically visible aggregates of protein. Their presence in tubular epithelium indicates an inability to efficiently transport resorbed proteins from the glomerular filtrate to the capillary blood at the abluminal surface. Two pathogenic mechanisms, either alone or in combination, may be responsible for droplet formation. The first mechanism involves direct toxic injury to the tubular epithelial cells, causing obstruction of protein transport and increased cytoplasmic accumulations. The other process results from glomerular disease in which excessive proteins leak into the glomerular filtrate and subsequently overwhelm the transport capacity of the tubular cells. To differentiate between these two possible mechanisms, electron microscopic studies were conducted in an effort to demonstrate glomerular lesions which might promote excess proteinuria. All structures, including the basement membrane, endothelial lining, and epithelial cell foot processes were normal in appearance. Over 190 ultrastructural photomicrographs of renal tissue were examined. Although these photographs confirmed the presence of increased cytoplasmic protein droplets in the proximal tubular epithelium and focal necrosis of tubular epithelium at the corticomedullary junction, there were no distinct morphologic changes observed in glomeruli.

The occurrence of renal lesions is quite high in the Fischer 344 rat strain. Coleman et al. (1977) detailed the incidence of pathologic changes during aging of Fischer 344 male rats. In all but one of 144 rats studied, some sort of renal pathology was

observed. There was a high correlation between increasing age and increasing severity of chronic nephropathy centered mainly on changes in the glomeruli. In the present study of decalin, there was an absence of glomerular involvement in the renal lesions observed in the male rats exposed to decalin, and the lesions appear to be distinctly different from those seen in cases of chronic nephropathy. This finding suggests that hyalin droplet formation and tubular epithelial cell necrosis observed at the conclusion of the 90-day exposure period were probably the result of the direct toxic effect of decalin or one of its metabolites.

In mice necropsied at the conclusion of the 90-day exposure, lesions that were considered to be dose-dependent were limited to the liver where 87% of the 5 ppm decalin exposure group and 94% of the 50 ppm decalin exposure group exhibited varying degrees of hepatocellular cytoplasmic vacuolization (fatty change). This lesion was present in only 6% of the unexposed control animals. Electron microscopic examination of the hepatocytes further indicated that increased cytoplasmic lipids were present in exposed animals and that these changes were accompanied by slight increases in smooth endoplasmic reticulum. Interpretation of ultrastructural findings, however, was largely subjective due to the relatively small sample size (3 exposed and 3 control mice) and the limited quantity of photomicrographs (33). It should be emphasized that fatty changes and increases in smooth endoplasmic reticulum are alterations which may result from a variety of toxic or metabolic insults.

The interim 19-month postexposure sacrifice of a portion of the rats occurred in early 1980. The mice were not sacrificed at that time because the number remaining after sacrifice would have been insufficient to warrant further holding. It was decided instead to hold all of the mice until 24 months on study at which time they, along with all remaining rats, were sacrificed. This sacrifice occurred in May 1980. Histopathologic examination of the tissues from the interim and final sacrifices has not been completed.

THE EXPERIMENTAL DETERMINATION OF THE ONCOGENIC EFFECTS OF PETROLEUM JP-4 VAPOR

In 1973-74, the chronic inhalation toxicity of JP-4 jet fuel was investigated by the Toxic Hazards Research Unit and reported in subsequent annual reports (MacEwen and Vernot, 1974, 1975, 1976). This study was conducted in Thomas Domes, 6 hours/day, 5 days/week for eight months, using rats, mice and dogs exposed to 5000 and 2500 mg/m³ petroleum JP-4 vapor concentrations.

Exposure to the high level, 5000 mg/m³, resulted in organ hypertrophy and bronchial irritation in rats and caused CNS effects and osmotic erythrocyte fragility increases in female dogs. The reason for the organ hypertrophy in rats was not clear

but appeared to be of little toxicologic significance as there was no tissue destruction or alteration seen on histopathologic examination. The increase in RBC osmotic fragility appears to have been a real effect of unknown etiology which was transient in nature. The central nervous system effect seen in dogs and respiratory irritation in rats are effects which could be considered relevant to possible human experience with chronic exposure to JP-4 vapor.

The exposure portion of a 90-day continuous study with rats, mice, and dogs has just been completed in our laboratory. This study was conducted in Thomas Domes at exposure concentrations of 1000 and 500 mg/m³. The dogs and one-third of the rodents were sacrificed at the conclusion of the exposure phase. The remaining rodents will be held a maximum of 24 months. The dogs in this study exhibited no CNS effects within the 90 days and no increases of red blood cell osmotic fragility were noted at these lower exposure concentrations. Tissue analysis is not yet available.

This study was designed to compare the tumorigenic potential of petroleum JP-4 fuel vapor with that from shale oil derived JP-4 fuels. Shale oil has been reported to be a more potent carcinogen when painted on mouse skin than petroleum oils.

Mice and rats are currently being exposed to JP-4 concentrations of 500 mg/m³ and 1000 mg/m³ by the inhalation route in Thomas Domes for one year using a work week schedule of 6 hours/day, 5 days/week with holidays and weekends excluded to simulate a human industrial exposure regimen. Each exposure group consists of 100 male and 100 female Fischer 344 rats and 100 male and 100 female C57B1/6 mice. Another group with the same numbers of animals is being held at the Veterinary Sciences Division Building (Vivarium) to serve as controls. Animals are caged in conformance with ILAR standards for laboratory animal care.

Following the exposure period, 10% of the rodents from each group will be sacrificed while the remaining rodents will be held for postexposure observation for one additional year or until cumulative mortality reaches 90%.

The generation of JP-4 vapor for the exposure chambers is being done in the same manner as previously described for the 90-day continuous exposure studies of petroleum JP-4 and the chambers are continuously monitored in the same manner.

All animals are being observed hourly during the exposure and will be observed daily thereafter until the mortality rate warrants more frequent examinations. At that time, cage group size will be reduced, and observations will be increased to 6/day

at 4-hour intervals. Animals found in a moribund condition are sacrificed. This is done to reduce cases of PMD and cannibalism as much as possible.

Rats are individually weighed at biweekly intervals during exposure and will be weighed monthly during the postexposure period. Mice will be weighed in groups with the group mean weights being followed on a monthly basis throughout the experimental period. Blood will be taken via the portal vein from the rats sacrificed at the exposure phase termination for the tests shown in Table 42.

TABLE 42. CLINICAL HEMATOLOGY AND CHEMISTRY TESTS
PERFORMED ON RATS EXPOSED TO PETROLEUM JP-4 VAPOR

<u>Hematology</u>	<u>Chemistry</u>
Hematocrit	Sodium
Hemoglobin	Potassium
RBC	Calcium
WBC	Albumin/Globulin
Differentials	Total Protein
Mean Corpuscular Volume (MCV)	Glucose
Mean Corpuscular Hemoglobin (MCH)	Alkaline Phosphatase
Mean Corpuscular Hemoglobin Concentration (MCHC)	SGPT
	SGOT
	Bilirubin
	Creatinine
	BUN

All animals that die or are sacrificed in these studies will be necropsied, external examination to include body orifices and fixation of 33 tissues using the NCI protocol for oncogenic screening. Liver, kidney, and spleen weights will be obtained for all rats examined at the scheduled sacrifice.

Exposures began on 11 February 1980 and will continue through February 1981 with postexposure observation ending in February 1982.

THE EXPERIMENTAL DETERMINATION OF SAFE ATMOSPHERIC EXPOSURE CONCENTRATIONS OF JP-10 VAPOR

The experimental protocol designed to establish safe exposure limits as well as to identify the oncogenic potential of JP-10 fuel can be found in a previous annual report (MacEwen and Vernot, 1979) which also contains information on the first 10 months of the planned 12-month industrial-type chronic exposure of four animal species to 100 ppm JP-10. Distribution of the

animal groups used in this study is shown below along with other pertinent information.

<u>Species</u>	<u>Sex</u>	<u>Strain</u>	100 ppm	JP-10	<u>Unexposed Controls</u>
			Chamber 5	Chamber 6	
Rats	Male	Fischer 344	-	50	50
Rats	Female	Fischer 344	-	50	50
Mice	Female	C57B1/6	200	-	200
Hamsters	Male	Golden Syrian	100	-	100
Dogs	Male	Beagle	-	4	4
Dogs	Female	Beagle	-	4	4

The exposure phase of the study was initiated in June 1978 and concluded 12 months later. Twenty exposed mice, 20 control mice, 10 exposed hamsters, and 10 control hamsters were sacrificed within a two-day period following cessation of exposure and submitted for gross and histopathologic examination to determine if reversible tissue changes were present at that time.

Mortality during the exposure phase of the study was limited and absent in many cases. The mortality ratios at exposure conclusion and at 10 months postexposure are shown in Table 43.

TABLE 43. MORTALITY RATIOS FOR GROUPS OF JP-10 EXPOSED AND CONTROL ANIMALS AT EXPOSURE CONCLUSION AND AT 10 MONTHS POSTEXPOSURE

<u>Species, Sex</u>	<u>Unexposed Controls</u>		<u>100 ppm JP-10 Exposed</u>	
	<u>Exposure Conclusion</u>	<u>10-Months Postexposure</u>	<u>Exposure Conclusion</u>	<u>10-Months Postexposure</u>
Mice, female	30/200	^a 114/200	20/200	^a 116/200
Rats, male	0/50	9/50	0/50	6/50
Rats, female	4/50	^a 16/50	0/50	^a 14/50
Hamsters, male	5/100	^a 40/100	9/100	^a 35/100
Dogs, male	0/4	0/4	0/4	0/4
Dogs, female	0/4	0/4	0/4	0/4

^a Includes a 10% sacrifice within 2 days of exposure termination.

Mean body weights for groups of exposed and control male rats, female rats, and male hamsters obtained on a biweekly schedule throughout 12 months of exposure and monthly through 10 months postexposure are shown in Figure 20. Weights of male rats and hamsters show depression as a result of JP-10 exposure. Values for male rats are statistically different from control values at all times during exposure and postexposure. Values for exposed hamsters are also statistically different from controls at all weighing periods during exposure. This pattern was continued during the postexposure phase.

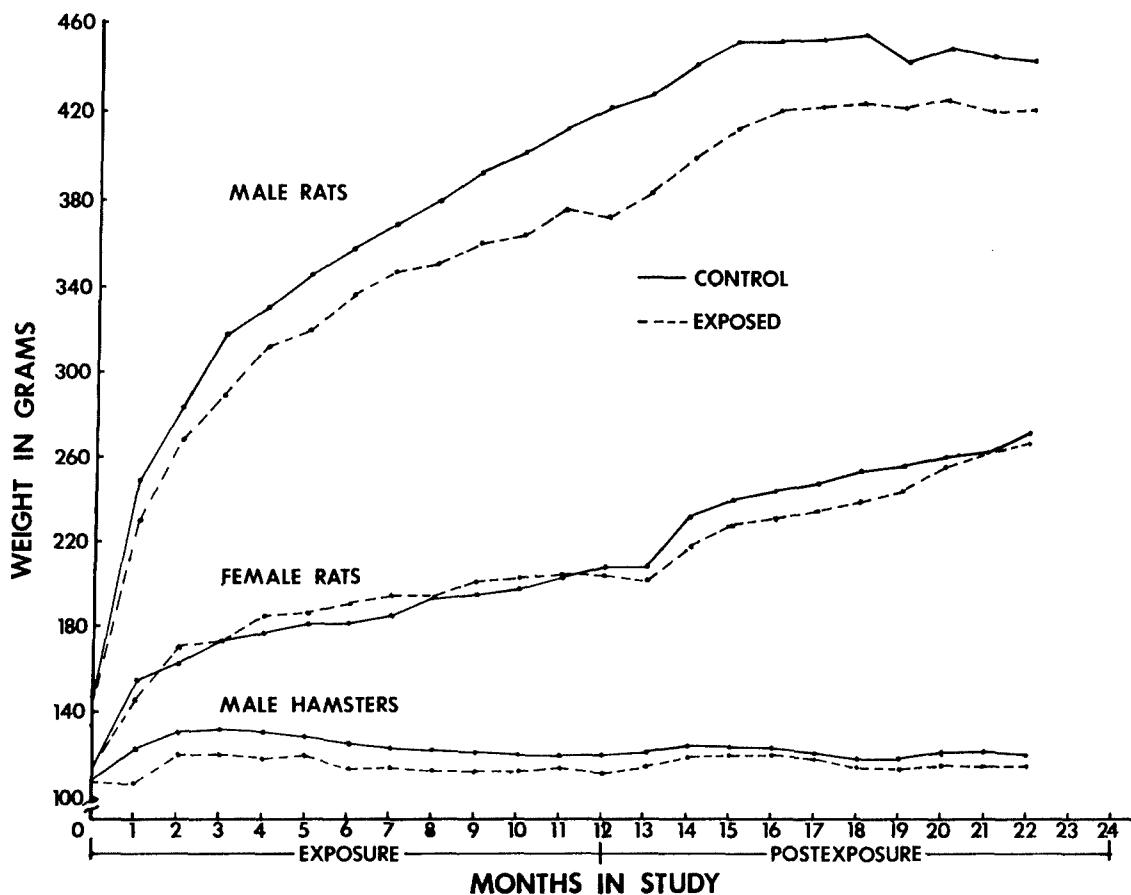


Figure 20. Mean body weights of rats and hamsters exposed intermittently to 100 ppm JP-10 for one year.

Exposed female rat weights are not significantly different from controls at any phase of the study. An examination of exposed dog and mouse weights taken during and after exposure thus far reveals no effect of JP-10 exposure.

An examination of the clinical chemistry values from the battery of tests conducted biweekly on dogs throughout the 52 weeks of exposure revealed nothing noteworthy except for total protein and globulin results. Albumin values for exposed dogs were stable and comparable with controls throughout the exposure. Slightly elevated protein values, therefore, led to slight elevations in the calculated globulin fraction from weeks 2 through 52. Statistical differences from control values were seen in 9 of 16 measurements from exposure week 22 to exposure conclusion but the albumin/globulin ratios for the exposed dogs were well within normal limits for this species. The results of postexposure quarterly physical examinations and semi-annual clinical chemistry measurements indicate that all exposed and control dogs are in good health. The dogs will be maintained until June 1984.

The two exposure chamber concentrations were monitored sequentially with a Beckman Model 400 hydrocarbon analyzer throughout the exposure period. The 12 monthly mean chamber concentrations of JP-10 are given in Table 44.

TABLE 44. JP-10 EXPOSURE MEAN MONTHLY CONCENTRATIONS
PRESENTED TO EXPERIMENTAL ANIMALS
(PARTS PER MILLION)

<u>Chamber 5</u>	<u>Chamber 6</u>
99.2	100.0
99.4	99.5
100.8	98.9
101.4	101.1
100.3	100.3
99.3	100.0
99.4	99.7
99.8	100.5
99.6	99.7
100.4	99.9
98.7	100.1
100.3	100.0

There is no pathology information available at this time and the postexposure observations are continuing.

A 12-MONTH CHRONIC INHALATION EXPOSURE OF ANIMALS TO METHYLCYCLOHEXANE TO DETERMINE ITS ONCOGENIC POTENTIAL

Methylcyclohexane (MCH) is a constituent of jet aircraft fuel JP-9. This fuel is a mixture of three primary ingredients, JP-10, RJ-5, and MCH. JP-10 and RJ-5 are high density hydrocarbons yielding a greater BTU output than conventional aircraft fuels. They also have high viscosity which causes pumping and flow problems at low temperature that are corrected by the addition of MCH to the mixture.

A six-month chronic inhalation exposure to RJ-5 conducted in our laboratory has been reported in the 1975 annual report (MacEwen and Vernot, 1975) as well as elsewhere in this report. Acute and sub-chronic toxicity studies of MCH have been reported by Treon et al. (1943). Six-hour acute exposures of rabbits to inhaled concentrations of MCH above 10,000 ppm caused convulsions, light narcosis, labored breathing, salivation, and conjunctival congestion. Between 5500 and 7300 ppm, lethargy and impaired coordination were the only signs.

Lazarew (1929) reported that 7500 to 10,000 ppm vapor for two hours produced narcosis in mice while 10,000 to 12,000 ppm caused death. Lehman and Flury (1943) indicated that the acute toxicity of MCH was greater than that of heptane, but less than that of octane. Similar high level narcotic effects were reported when mice were exposed to heptane vapor between 10,000 and 15,000 ppm (Fuehner, 1921). In addition, Patty and Yant (1929) reported slight dizziness in man after exposure to 1000 ppm for six minutes. Concentrations of 1000 to 5000 ppm resulted in marked vertigo, nausea, incoordination, and hilarity which persisted for several hours after exposure.

In 1976, the American Conference of Governmental Industrial Hygienists lowered the threshold limit value (TLV) for MCH from 500 ppm to 400 ppm or 1600 mg/m³. The recommended short-term exposure limit (STEL) is 500 ppm or 2000 mg/m³. These values are based on analogy to the toxicity of heptane and are identical to the TLV and STEL of heptane.

The scarcity of chronic exposure data for animals and the consequent use of analogy to other solvents for setting human exposure limits is risky. Prolonged exposure to methylbutylketone and n-hexane have been shown to cause peripheral polyneuropathy in man (Billmaier, 1974 and Allen, 1975). A TLV of 500 ppm had been set for n-hexane based solely on acute toxicity data and comparison with other petroleum solvents such as pentane. Reports of neuropathy in workers exposed to hexane resulted in the lowering of the American Conference of Governmental Industrial Hygienists TLV to 100 ppm in 1977.

These studies were undertaken to obtain the data needed to assess the safety margin of current exposure limits for methylcyclohexane.

Animal exposure concentrations of MCH for this study were selected on the basis of the current TLV (400 ppm) and 2000 ppm which appeared to be a maximum tolerated level for repeated exposures.

The exposure portion of this study began on 1 August 1978 and continued for one year after which 20 mice, 10 rats, and 10 hamsters from each group were necropsied to assess chronic toxicity effects in primary tissues. The remaining rodents and dogs

are being held for an additional year of observation or until the cumulative mortality in any subgroup of a species reaches 90%. Each exposure and control group of animals consisted of 65 male and 65 female rats, 200 female mice, 100 male hamsters, and 8 dogs equally divided by sex. The numbers of rodents used were selected to provide a statistically valid number of each sex and species which had reached the required age for tumor induction allowing for natural and toxicologic attrition.

Two large chambers were used for each exposure level to house the animals in a manner compatible with good animal care practices. Rats and dogs were exposed in one chamber, and a companion chamber receiving the same MCH concentration housed the mice and hamsters.

The one-year inhalation exposure of animals to MCH simulated an industrial work week schedule of 6 hours/day, 5 days/week with holidays and weekends off. The animals had food available ad libitum during the nonexposure periods but it was removed from the chamber during exposure. Cleaning and feeding was done following the daily exposure period.

The animals used in this study are observed at least 6 times daily during the postexposure holding phase of the study. Rats, hamsters, and dogs were weighed individually at biweekly intervals during exposure and are being weighed monthly during the postexposure period. Mice are weighed in groups with group mean weights followed on a monthly basis throughout the experimental period.

Blood samples are drawn from all dogs at biweekly intervals and clinical determinations performed for a battery of tests including routine hematology tests, electrolytes, glucose, creatinine, bilirubin, serum protein, albumin, and three enzymes, SGPT, SGOT, and alkaline phosphatase. The same clinical hematology and blood chemistry tests done routinely on the dogs were also done on blood from the rats necropsied at the end of the one-year exposure. Organ weight data were also obtained and evaluated for these animals. All of the animals used in this study are necropsied at death with a battery of approximately 33 tissues sampled for histopathology examination following the protocol used by the National Cancer Institute.

The generation of the desired concentration of methylcyclohexane was described in the last annual report (MacEwen and Vernot, 1979). Chamber concentrations were monitored with a total hydrocarbon analyzer which analyzed the concentrations of MCH from each pair of chambers on a 15-minute cycle.

The exposure concentrations achieved during the 12-month exposure period are shown in Table 45. Exposure concentration control was excellent after the first few weeks and is reflected in the relatively small standard deviations of the means.

TABLE 45. MEAN METHYLCYCLOHEXANE CONCENTRATIONS
MEASURED IN ANIMAL EXPOSURE CHAMBERS

	Chamber 1	Chamber 2	Chamber 3	Chamber 4
No. of Sampling Days	243.0	243.0	243.0	243.0
Nominal Conc., ppm	400.0	400.0	2000.0	2000.0
Mean Measured Conc., ppm	401.5	398.9	2008.7	1997.7
Standard Deviations, ppm	+4.50	+2.45	+46.57	+52.37
Concentration Range, ppm	393-412	395-402	1878-2080	1847-2047

Purity analyses have been made on each drum of methylcyclohexane used in the study. The drums contained 3 different lots of MCH manufactured by Eastman Organic Chemical Corporation and were designated as lots A8, A9 and B8. Lot A8 contained 98.57% MCH with two impurities, 0.86% n-heptane and 0.56% toluene, while lot A9 was 98.50% pure containing 0.97% n-heptane and 0.52% toluene. Lot B8 contained 98.66% MCH, 0.74% n-heptane and 0.60% toluene. Only the two impurities were identified and the relative purity of the MCH was consistent from drum to drum within lots and between lots used in the study.

Mean body weights of MCH exposed and control rats are shown in Figure 21. The female rat weights were unaffected during exposure as well as during the postexposure observation period. MCH exposed male rats show a growth depression during the exposure portion of the study and although they gained weight after removal from the exposure chambers, they still have not attained the mean weight of the unexposed control group. A definite depression in mean body weights is seen in the exposed hamster groups (Figure 22). Immediately following exposure, both exposed groups gained weight and became equivalent to the control group.

Clinical determinations on dog blood taken at biweekly intervals have given variable but non-MCH related results to date. The last annual report (MacEwen and Vernot, 1979) noted transient dose-related increases in SGPT levels which were caused by a single dog per group exhibiting high levels while the remainder of the group were normal. This difference was not seen after 29 weeks of exposure. Postexposure blood values are within the expected normal limits.

The mean organ weights for randomly chosen male and female rats sacrificed at the conclusion of the exposures are shown in Table 46. The statistically significant effects seen in the mean organ weights or their ratios to body weight do not appear to be of any biological significance. The mean heart weight of the female rats from the low test group is significantly higher than controls while the mean weight of this organ in the high test group is lower than controls. The mean heart weight of the male test rats compared favorably with their respective control groups. The ratio of heart to body weight of the high test male

rats was statistically higher than the controls, while the female ratio was significantly lower. Similar results can be seen when comparing the difference in mean liver weights of the groups.

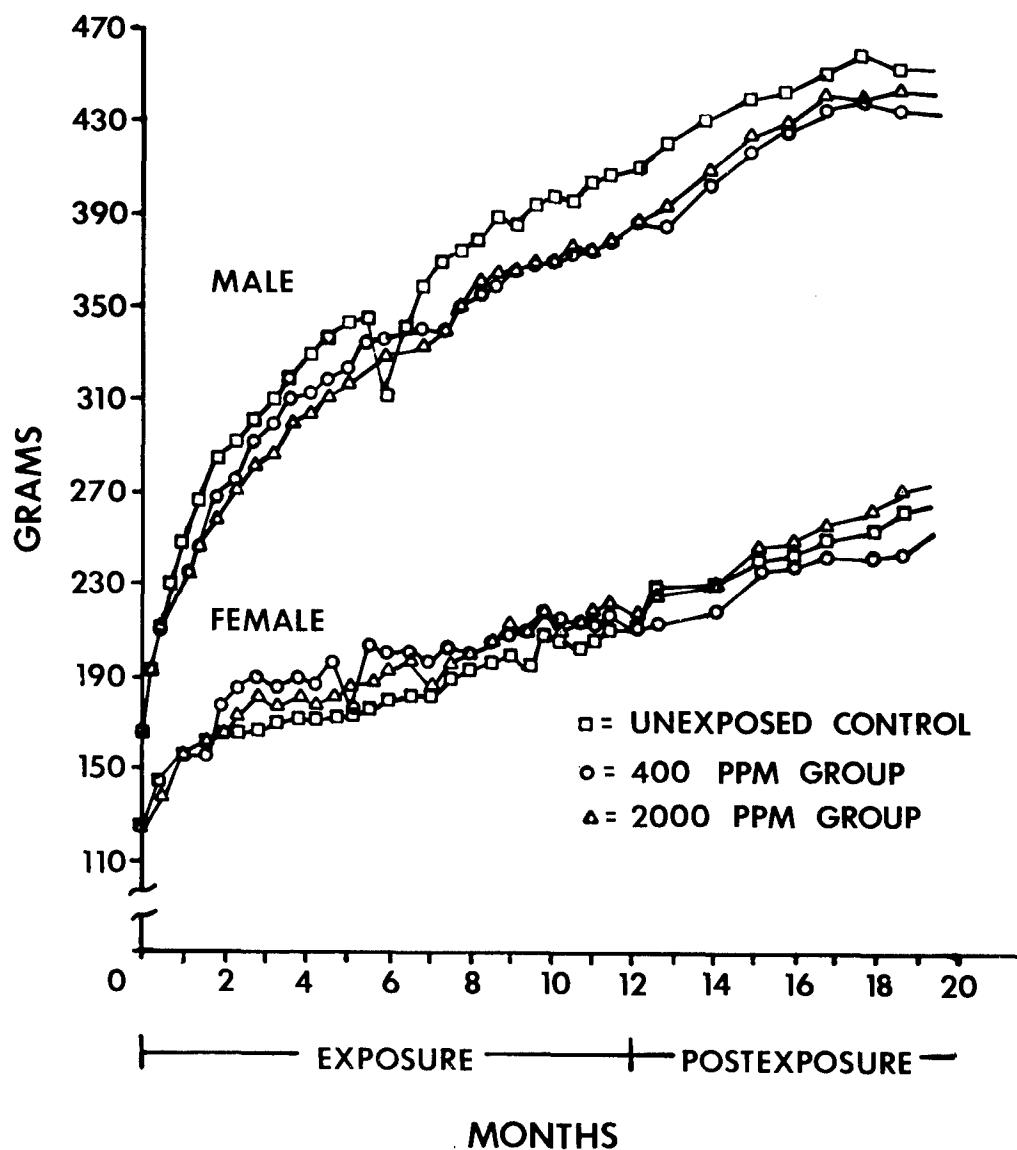


Figure 21. Effect of methylcyclohexane exposure on Fischer 344 rat body weight.

The hematology and clinical chemistry values of the male rats sacrificed after one year of exposure are shown in Table 47 and the hematology values for the female rats are listed in Table 48. The male rats show a dose related decrease in hemoglobin and sodium. A significant increase in creatinine along with an increase in BUN was seen in the low test male group but not evident in the high male test group. Low WBC's were found in all test groups, both male and female.

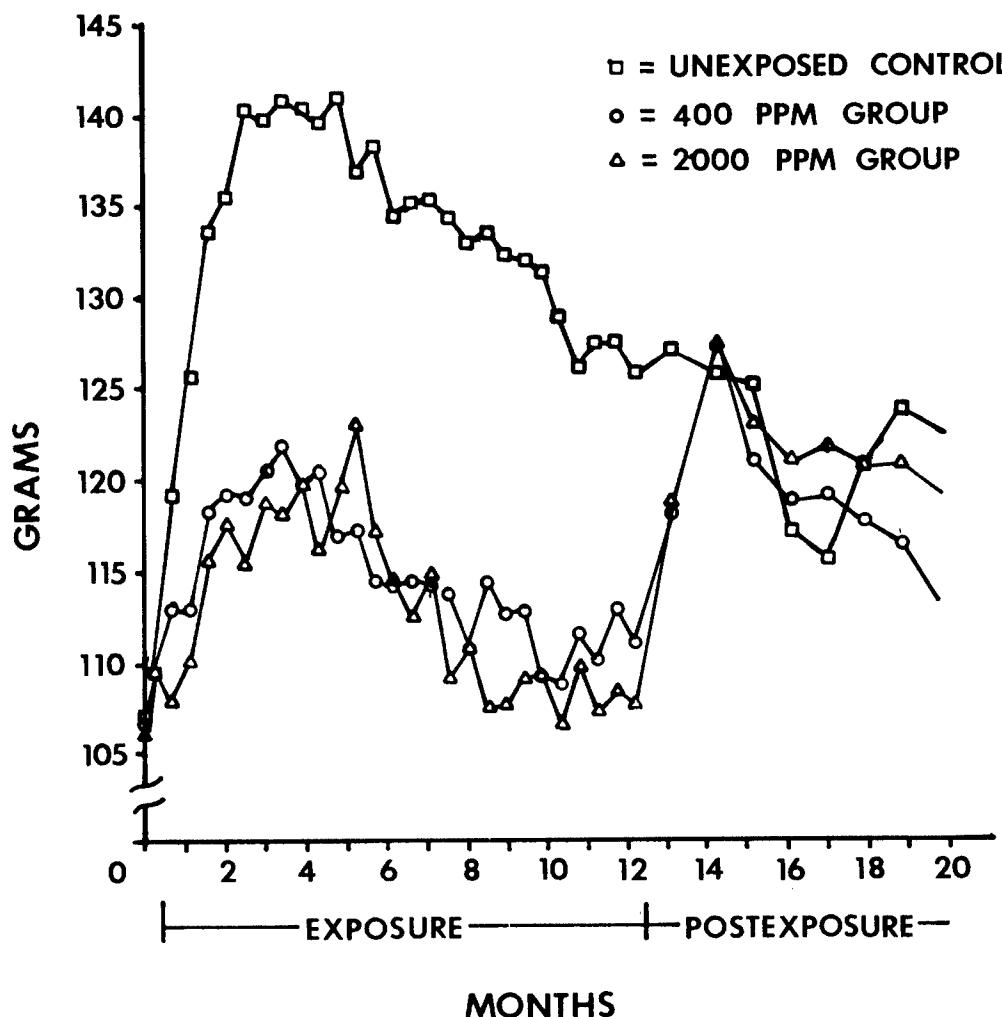


Figure 22. Effect of inhalation exposure to methylcyclohexane in golden syrian hamster body weight

Because of hemolysis in most samples of female rat blood, no clinical chemistry comparisons could be made. The males had only 1 of 29 samples hemolyzed.

A male dog from the low test group died on 11 February 1980, seven months postexposure. This dog showed no signs of physical problems and appeared normal when observed that morning. During the afternoon observation, the dog was found dead. Gross examination revealed an acute intestinal problem. There was an annular constriction in the intestine which caused severe transmural congestion and hemorrhage. All of this resulted in peritonitis and death. It is highly unlikely that this was an MCH exposure related death.

TABLE 46. RAT ORGAN WEIGHTS^a MEASURED AT THE CONCLUSION OF ONE-YEAR EXPOSURE TO METHYLCYCLOHEXANE

	<u>Unexposed Controls</u>	<u>400 ppm</u>	<u>2000 ppm</u>
<u>Male Rats</u>	(N=10)	(N=9)	(N=10)
Body weight, gms.	391.8 \pm 12.7	369.9 \pm 21.3 ^b	362.1 \pm 17.3 ^c
Heart wt., gms.	1.04 \pm 0.08	1.01 \pm 0.16	1.08 \pm 0.8
Heart/100 g body wt.	0.27 \pm 0.03	0.27 \pm 0.04	0.30 \pm 0.02 ^b
Lung wt., gms.	1.29 \pm 0.14 ^e	1.18 \pm 0.11	1.15 \pm 0.12 ^b
Lung/100 g body wt.	0.33 \pm 0.04 ^e	0.32 \pm 0.03	0.32 \pm 0.04
Liver wt., gms.	9.60 \pm 0.76	9.41 \pm 1.03	10.21 \pm 0.78
Liver/100 g body wt.	2.45 \pm 0.22	2.54 \pm 0.18	2.82 \pm 0.12 ^{c,d}
Spleen wt., gms.	0.64 \pm 0.20	0.57 \pm 0.09	0.63 \pm 0.08
Spleen/100 g body wt.	0.16 \pm 0.05	0.15 \pm 0.02	0.17 \pm 0.02
Kidney wt., gms.	2.48 \pm 0.12	2.39 \pm 0.21	2.39 \pm 0.11
Kidney/100 g body wt.	0.63 \pm 0.05	0.65 \pm 0.03	0.66 \pm 0.02
<u>Female Rats</u>	(N=10)	(N=8)	(N=10)
Body wt., gms.	203.2 \pm 10.6	203.8 \pm 9.3	201.5 \pm 7.7
Heart wt., gms.	0.69 \pm 0.03	0.74 \pm 0.05 ^b	0.64 \pm 0.11 ^d
Heart/100 g body wt.	0.34 \pm 0.02	0.36 \pm 0.04	0.32 \pm 0.05 ^d
Lung wt., gms.	0.86 \pm 0.11	0.86 \pm 0.09	0.80 \pm 0.05
Lung/100 g body wt.	0.43 \pm 0.07	0.42 \pm 0.05	0.40 \pm 0.02
Liver wt., gms.	5.11 \pm 0.43	4.85 \pm 0.32	4.46 \pm 0.36 ^{c,d}
Liver/100 g body wt.	2.52 \pm 0.19	2.39 \pm 0.17	2.22 \pm 0.19 ^c
Spleen wt., gms.	0.44 \pm 0.05	0.48 \pm 0.07	0.41 \pm 0.06 ^d
Spleen/100 g body wt.	0.22 \pm 0.02	0.23 \pm 0.04	0.20 \pm 0.02 ^d
Kidney wt., gms.	1.44 \pm 0.07	1.48 \pm 0.12	1.39 \pm 0.07 ^d
Kidney/100 g body wt.	0.71 \pm 0.05	0.73 \pm 0.06	0.69 \pm 0.05

^a Mean \pm S.D.

^b Different from controls at the 0.05 level of significance.

^c Different from controls at the 0.01 level of significance.

^d Different from the other test group at the 0.05 level of significance

^e N=9, this parameter

TABLE 47. MEAN HEMATOLOGY AND CLINICAL CHEMISTRY VALUES OF MALE RATS AFTER A ONE-YEAR INHALATION EXPOSURE TO MCH VAPOR

Parameter	Control	N	400 ppm	N	2000 ppm	N
RBC (10^6)	9.7	10	9.8	9	9.7	10
WBC (10^3)	6.7	10	5.4 ^a	9	5.3 ^a	10
HCT (%)	47.7	10	48.9 ^{a,d}	9	47.0	10
HGB (gm/dl)	15.2	10	15.4	9	14.7 ^{b,d}	10
Total Pro. (gm/dl)	7.2	10	7.3	9	7.3	9
Albumin (gm/dl)	4.2	10	4.2	9	4.1	9
Globulin (gm/dl)	3.0	10	3.1	9	3.1	9
Glucose (mg/dl)	162.8	10	170.3	9	165.8	9
Potassium (mEq/L)	5.3	10	6.0	9 ^{a,c}	5.4	9
Calcium (mg/dl)	9.6	10	9.7	9	10.5	9
Sodium (mEq/L)	154.9	10	151.6 ^b	9	150.1 ^b	9
Bilirubin (mg/dl)	0.38	10	0.40	9	0.38	9
BUN (mg/dl)	14.2	10	15.4	9	14.4	9
Creatinine (mg/dl)	0.55	10	0.64 ^{b,d}	9	0.58	9
SGPT (IU/l)	62.8	10	60.9	9	58.2	9
SGOT (IU/l)	91.6	10	94.7	9	86.4	9
Alk. Phos (IU/l)	12.5	10	11.8	9	9.7	9

^a Significantly different from controls, $p < 0.05$.

^b Significantly different from controls, $p < 0.01$.

^c Significantly different from other test group, $p < 0.05$.

^d Significantly different from other test group, $p < 0.01$.

TABLE 48. MEAN HEMATOLOGY VALUES OF FEMALE RATS AFTER A ONE-YEAR INHALATION EXPOSURE TO MCH VAPOR

Parameter	Control	N	400 ppm	N	2000 ppm	N
RBC (10^6)	7.8	10	7.8	10	7.9	10
WBC (10^3)	5.4	10	4.8	10	3.6 ^a	10
HCT (%)	44.1	10	43.1	10	44.0	10
HGB (gm/dl)	14.5	10	14.4	10	14.3	10

^a Significantly different from controls, $p < 0.01$.

THE EXPERIMENTAL DETERMINATION OF SAFE ATMOSPHERIC EXPOSURE CONCENTRATIONS FOR RJ-5

RJ-5 is a high density hydrocarbon found in the jet aircraft fuel JP-9. This fuel is a mixture of three primary ingredients, JP-10, methylcyclohexane, and RJ-5. RJ-5 yields a greater BTU output than conventional fuels but because of the high viscosity, pumping and flow problems occur at low temperatures. This problem is corrected by the addition of the methylcyclohexane to the fuel mixture.

A 6-month chronic inhalation toxicity exposure to 0.15 mg/liter RJ-5 was conducted with animals in our laboratory and reported by MacEwen and Vernot in 1975. A subnormal weight gain was noted in rats and particularly in dogs during the course of the experiment. Dogs and rats (CFE strain) sacrificed immediately following the conclusion of the exposures showed acute inflammation of the lungs as well as several cases of bronchopneumonia in the test groups.

A high incidence of alveolargenic carcinomas was seen in the mice held one-year postexposure following the 6-month exposure to 0.15 mg/liter RJ-5. The mice used in that study were of the CF-1 strain which is predisposed to this type of tumor. To determine if this compound truly possesses oncogenic properties, it was decided to do a more in-depth study for a longer time and to maintain a greater number of animals during the postexposure observation period. The rats and mice being used in this study are the strains which have been used in all of our recent oncological studies, Fischer 344 and C57B1/6, respectively.

RJ-5 is a mixture of stereoisomers of the reduced dimer of bicycloheptadiene containing six major components. Some of the physical properties are listed below:

Empirical Formula:	C ₁₄ H ₂₀
Molecular Weight:	188
Boiling Range (° F):	500-525
Vapor Pressure (70° F):	0.25 mm Hg
Density (70° F):	1.0813 g/ml

Rats, mice, hamsters, and dogs are exposed to 0.03 and 0.15 mg/liter vapor by the inhalation route in exposure chambers for one year using an industrial work week exposure of 6 hours/day, 5 days/week with holidays and weekends off to simulate a human work regimen.

Following the exposure period, 20 mice, 10 rats, and 10 hamsters per group will be sacrificed while the remaining rodents will be held postexposure for an additional year of observation or until cumulative mortality reaches 90%. The dogs will be held for postexposure observation for one year during which time they will receive quarterly physical examinations and blood analyses.

Purebred beagle dogs being used in this study have been provided by the Air Force. Rodents being used are listed below:

<u>Species</u>	<u>Strain</u>	<u>Source</u>
Rats	Fischer 344	Charles River Breeding Labs
Mice	C57B1/6	Jackson Laboratory
Hamsters	Golden Syrian	Charles River Breeding Labs

The animals have food available during the nonexposure periods, and cage areas are cleaned daily following completion of the 6-hour exposure and minimum 30-minute air purge.

Each chamber contains as few species as possible to minimize the risk of cross infection. Therefore, dogs and rats are housed in one chamber and the mice and hamsters in a companion chamber. The number of animals in each chamber is compatible with ILAR standards for animal care. The numbers of rodents permit a statistically valid number for each species to reach the required age for tumor induction with natural and toxicologic attrition. The chamber animal load is shown in Table 49.

TABLE 49. ANIMAL DISTRIBUTION IN RJ-5 EXPOSURE CHAMBERS

<u>Chamber Number</u>	Concentration, mg/liter					<u>Unexposed Controls</u>
	<u>0.03</u>	<u>0.03</u>	<u>0.15</u>	<u>0.15</u>	<u>0</u>	
<u>Species and Sex</u>						
Rats, male	-	65	65	-	65	
Rats, female	-	65	65	-	65	
Mice, female	200	-	-	200	200	
Hamsters, male	100	-	-	100	100	
Dogs, male	-	4	4	-	4	
Dogs, female	-	4	4	-	4	

RJ-5 vapor generation is done separately for each chamber. A Buchler polystaltic pump with viton tubing is used to pump the liquid RJ-5 into a stainless steel introduction line heated by a one-inch Glas-Col heating tape. The RJ-5 vapor is introduced into the chamber air supply line by the slight negative pressure in the intake air stream.

The chamber RJ-5 concentrations are monitored with a hydrocarbon analyzer. The analyzer is calibrated weekly using known concentrations of RJ-5 vaporized in Mylar bags. Sequential sampling is conducted on each pair of chambers.

All animals are observed hourly during exposure and will be observed daily thereafter until termination of each experiment.

Rats, hamsters, and dogs are being weighed individually at biweekly intervals during exposure and will be weighed every four weeks during the postexposure period. Mice are weighed in groups with group mean weights followed on a monthly basis throughout the experiment.

Clinical hematology and blood chemistry determinations will be made on blood taken from the rats sacrificed at termination of exposure. Organ weights (lung, liver, heart, kidney, and spleen) will be recorded for these rats.

Blood samples are drawn from all dogs at biweekly intervals and clinical determinations made for the following battery for tests:

HCT	Potassium
HGB	Calcium
RBC	Albumin/Globulin
WBC	Total Protein
Differentials	Glucose
MCV	Alkaline Phosphatase
MCH	SGPT
MCHC	SGOT
Sodium	Bilirubin
BUN	Creatinine

All animals that die in these studies are necropsied. If cannibalism or autolysis precludes the examination, a completed record containing this information is filed.

Exposures began during October 1979 and have been in progress approximately seven months at the time this report was prepared. Very few signs of toxic stress have been observed since the beginning of the animal exposures. Exposed mouse and dog weights are unaffected thus far. Mean body weights for groups of exposed and control male and female rats measured on a biweekly schedule through 22 weeks of study are shown in Figure 23. Included in this figure are the mean body weights of the control and test male hamsters through 21 weeks of the study. Mean body weights of the male rats are depressed as a result of the exposure. The female rats do not show this effect on mean body weight but are actually gaining weight at a rate greater than the female control group. The growth rate of the RJ-5 exposed hamsters is depressed in the same manner as the male rats.

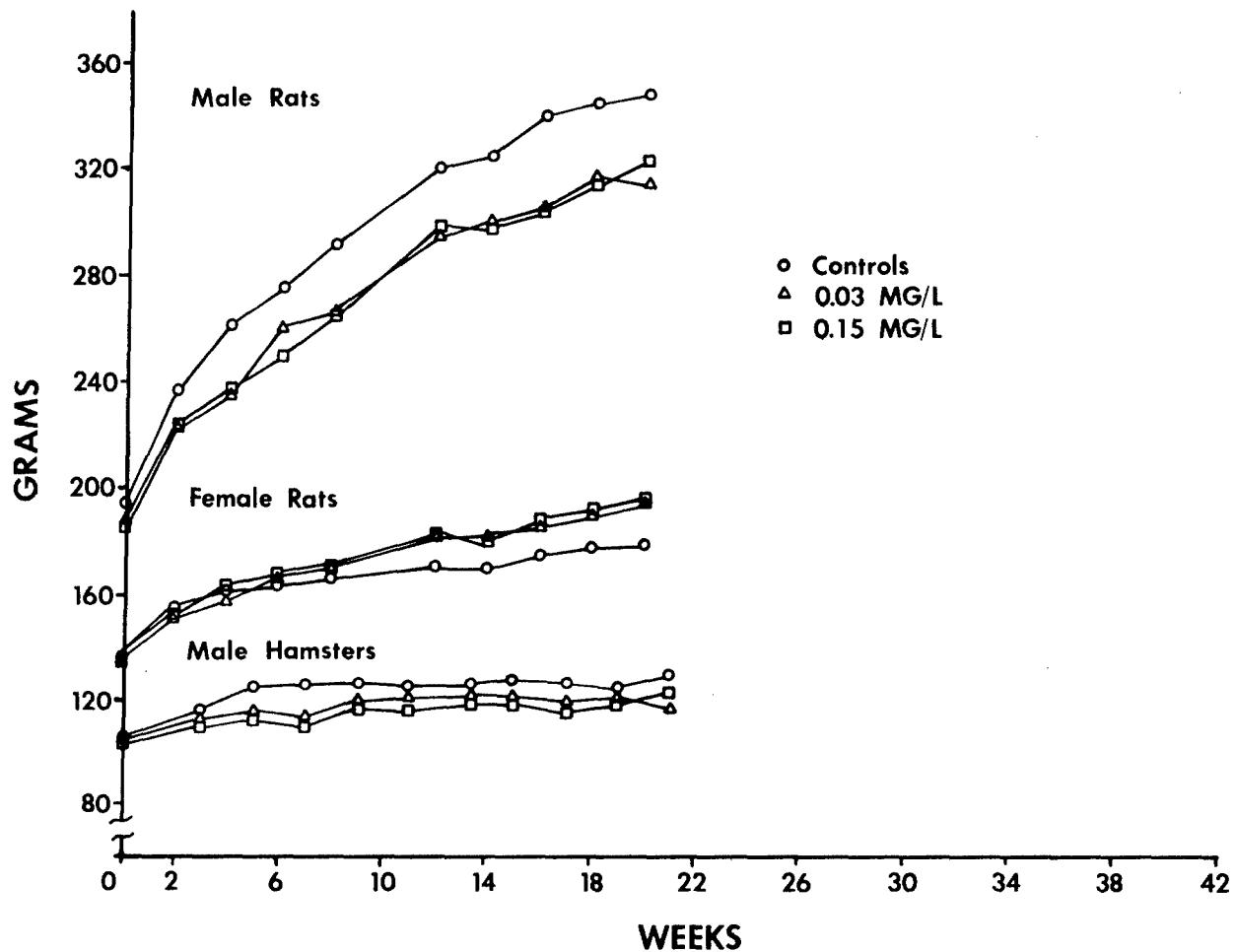


Figure 23. Mean body weights of rats and hamsters exposed intermittently to RJ-5 vapor.

Various clinical chemistry values measured on the dogs through 20 weeks of exposure have shown occasional statistical differences. However, they are not biologically significant, and no trend to adverse hematologic effect is seen.

Mortality has been limited in most animal groups and no deaths have been attributed to RJ-5 exposure. Ten control mice were accidentally drowned when a water valve stuck and filled their holding cage.

The exposures are to be completed in October 1980 and post-exposure observation begun at that time. The study will continue until October 1981.

CHRONIC EFFECTS OF LOW LEVEL INHALATION EXPOSURES TO FLUOMINE PARTICULATES

The compound fluomine [cobalt-bis (3-fluorosalicylaldehyde)-ethylene diimine], when activated, is capable of selectively absorbing oxygen from the air and upon heating will release pure molecular oxygen. This oxygen-scavenging property renders it useful as a possible component in life support systems for high altitude aircraft flights.

Preliminary studies of two-week duration as well as 6-month chronic inhalation exposures were described in previous annual reports (MacEwen and Vernot, 1977, 1978, and 1979). Included in the earlier reports were experimental data obtained during a 6-month exposure of animals to 0.1 and 0.5 mg/m³ fluomine. Also included were data concerning the gross pathology observations on animals sacrificed immediately following exposure as well as mean body weight effects on animals held postexposure. The animal groups for each exposure concentration and controls consisted of 100 male Sprague-Dawley rats, 140 female CF-1 mice, 24 male Hartley guinea pigs, and 8 beagle dogs. The exposures were conducted 6 hours/day, 5 days/week.

The fluomine particulates, produced by a Wright Dust Feeder®, were generated into a 200 liter mixing chamber prior to being drawn into the exposure domes by negative pressure. Regulation of the dust feeder gear ratios and/or the air passing through the mixing chamber controlled the concentration as well as the particle size entering the chamber.

Analysis of the fluomine concentration was accomplished by taking hourly filter samples for colorimetric analysis. The fluomine samples were dissolved in 1N HNO₃ and absorbance at 365 nm measured using a GCA McPherson spectrophotometer. Checks were made by counting the particles in the 1.4 - 3.0 micron range using the Royco® analyzer. Since the greater part of the mass of the fluomine was in this size range, fluctuations in chamber concentrations could be easily detected by changes in the channel output representing this range.

An interim sacrifice of animals took place 12 months post-exposure, at which time all surviving guinea pigs and one-half of the surviving rats and mice were sacrificed. The remaining animals were held for an additional six months and then sacrificed. The numbers of rats and mice alive at the conclusion of the study are shown below:

<u>Fluomine Concentration (mg/m³)</u>	<u>Male Rats</u>	<u>Female Mice</u>
0.5	15	41
0.1	11	35
0.0 (Controls)	13	29

Gross examination of the rats, mice and dogs sacrificed immediately postexposure failed to reveal any exposure-related lesions. The only significant changes in the dog organs examined by light microscopy were a higher incidence of diffuse, mild pulmonary congestion with intraaveolar edema and greater severity of hydropic degeneration in the livers of the 0.5 mg/m³ group. The significant findings are shown in Table 50.

TABLE 50. SIGNIFICANT HISTOPATHOLOGY FINDINGS IN DOGS IMMEDIATELY AFTER 6-MONTH EXPOSURE TO FLUOMINE AEROSOLS

<u>Organ</u>	<u>Concentration, mg/m³</u>		
	<u>0.5</u>	<u>0.1</u>	<u>0.0</u>
Lung:			
Congestion, diffuse, mild	4	0	0
Intraaveolar edema	4	0	0
Liver:			
Vacuolation, hydropic, minimal	0	0	7
Vacuolation, hydropic, mild	2	4	0
Vacuolation, hydropic, moderate	3	0	0
Vacuolation, hydropic, severe	3	1	0

No unusual gross lesions were detected in any of the rodent groups, either in individuals that died or those sacrificed at any period. Except for tumors which will be dealt with later in this report, no significant micropathologic lesions were found in the mice from any sacrifice group. Incidental lesions were randomly distributed with no dose related responses noted.

Chronic inflammation of the trachea was seen in 70% of both groups of exposed rats on histopathologic examination immediately following exposure. No control rats showed this lesion of the trachea at this examination period. Rats that died or were sacrificed during the remainder of the experimental holding period did not evidence any higher incidence of tracheitis than that shown by control animals (Table 51).

TABLE 51. INCIDENCE OF CHRONIC INFLAMMATION OF TRACHEA IN RATS IMMEDIATELY FOLLOWING EXPOSURE TO FLUOMINE DUST

<u>Sample Period</u>	<u>Incidence</u>		
	<u>0.5 mg/m³</u>	<u>0.1 mg/m³</u>	<u>Control</u>
Conclusion of Exposure	7/10 ^a	8/22 ^{a(1)}	0/10
0-12 Months Postexposure	25/53	22/54	23/56
12-18 Months Postexposure	17/37	13/34	16/34
TOTALS	49/100	43/100	39/100

(1) Includes two rats that died during exposure.

^a Different from controls at the 0.01 level of significance [Fisher Exact Test (Bliss, 1967)].

The lungs of all rats sacrificed following exposure were carefully excised and weighed to give the values shown in Table 52. As can be seen, the mean wet lung weights of the exposed animals did not differ significantly from those of the control rats.

TABLE 52. EFFECT OF 6-MONTH INHALATION EXPOSURE TO FLUOMINE DUST ON RAT LUNG WEIGHT

<u>Fluomine Conc., mg/m³</u>	<u>Body Weight (grams)</u>	<u>Lung Weight (grams)</u>	<u>Lung/Body Wt. Ratio</u>
0.5	472.3	2.67	0.565
0.1	497.1	2.47	0.497
0.0	522.9	2.56	0.495

In addition to the chronic inflammation of the trachea seen in the rats examined immediately following exposure, bronchopneumonia was found in 17% of the rats from the 0.5 mg/m³ group which either died during the 12-month postexposure period or were sacrificed at 12-months postexposure (Table 53). Bronchopneumonia was not seen in any of the control rats examined during this time period nor was it noted in any of the rats at the other examination periods.

Histopathologic examination of the tissues removed from the guinea pigs at the 12-month postexposure sacrifice period showed no exposure related lesions. No tumors were found nor were any nonneoplastic lesions which could be considered treatment related found in any of the guinea pigs examined.

There were no significant differences in the numbers or types of tumors seen in the exposed rats when compared to the control group at any of the examination periods.

TABLE 53. BRONCHOPNEUMONIA IN RATS EXPOSED TO FLUOMINE DUST

<u>Sample Period</u>	<u>Incidence</u>		
	<u>0.5 mg/m³</u>	<u>0.1 mg/m³</u>	<u>Control</u>
Conclusion of Exposure	0/10	0/12 ⁽¹⁾	0/10
0-12 Months Postexposure	9/53	1/54	0/56
12-18 Months Postexposure	0/37	1/34	0/34
TOTALS	9/100	1/100	0/100

(1) Includes two rats that died during exposure.

As shown in Table 54, there was no increase in alveolar/bronchiolar adenomas in the high level (0.5 mg/m^3) mice that died between the 12th and 18th month or were sacrificed at 18 months postexposure. No differences can be found for this particular lesion at any of the other sampling periods (Table 55).

TABLE 54. ALVEOLAR/BRONCHIOLAR CARCINOMAS IN MICE EXPOSED TO FLUOMINE DUST

<u>Sample Period</u>	<u>Incidence</u>		
	<u>0.5 mg/m³</u>	<u>0.1 mg/m³</u>	<u>Control</u>
During Exposure ^a	0/22	0/38	0/23
0-12 Months Postexposure	4/66	0/58	3/71
12-18 Months Postexposure	2/52	7/44	6/46
TOTALS	6/140	7/140	9/140

^a Includes fourteen animals sacrificed at termination of exposure period.

TABLE 55. ALVEOLAR/BRONCHIOLAR ADENOMAS IN MICE EXPOSED TO FLUOMINE DUST

<u>Sample Period</u>	<u>Incidence</u>		
	<u>0.5 mg/m³</u>	<u>0.1 mg/m³</u>	<u>Control</u>
During Exposure ^a	0/22	0/38	0/23
0-12 Months Postexposure	15/66	11/58	17/71
12-18 Months Postexposure	21/52	13/44	13/46
TOTALS	36/140	24/140	30/140

^a Includes fourteen animals sacrificed at termination of exposure period.

Fluomine exposed rats showed a statistically significant depression in mean body weight gain (Figure 24) throughout the study. Although the test rat groups differed from the control groups, they rarely differed from each other and a dose-response was not established. Following transfer to laminar flow animal rooms, the rat groups showed a slight increase in mean body weight gain. Although all groups showed this increase, the test groups remained below the control group.

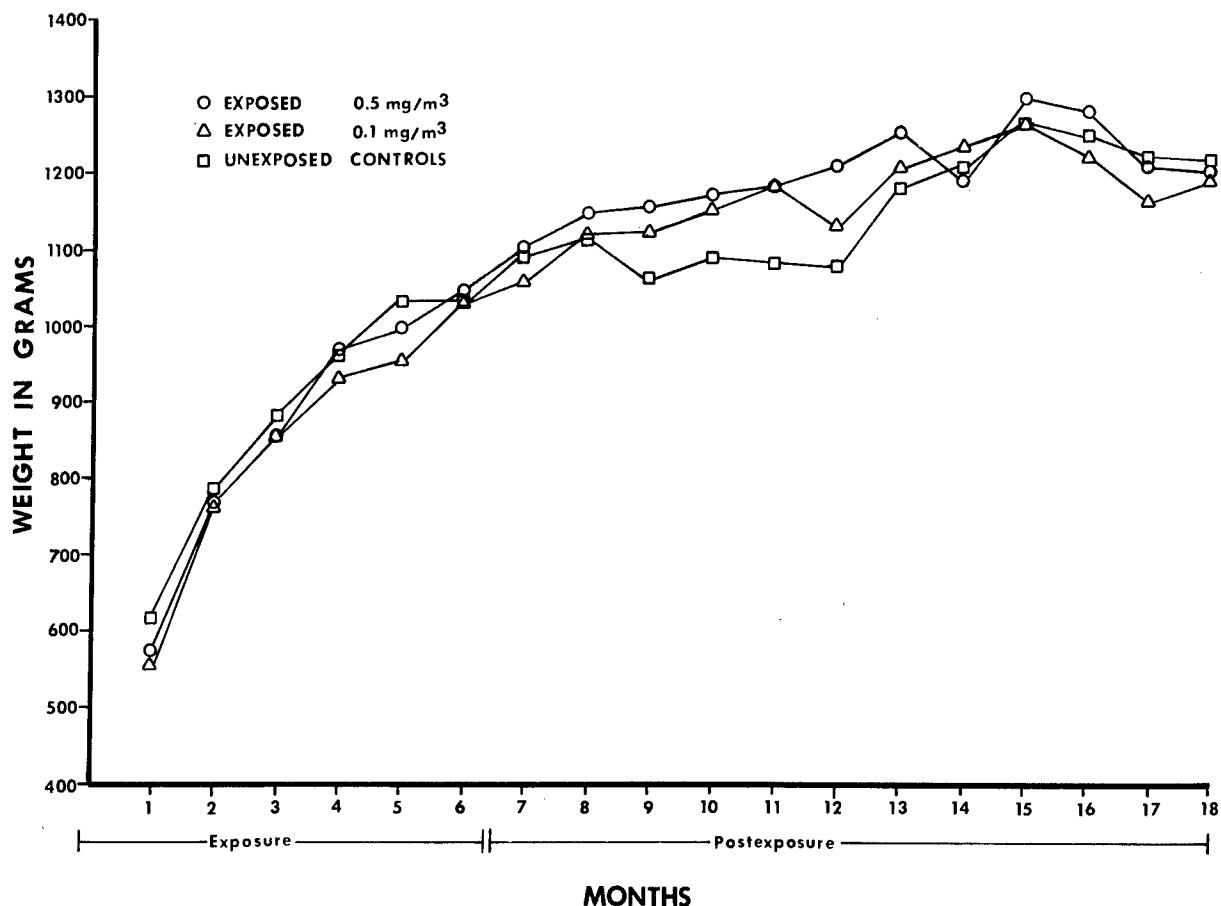


Figure 24. Effect of fluomine dust inhalation on male rat body weight.

The guinea pig mean body weights did not differ significantly from the control group during the exposure phase except for short periods of successive weighings as shown in Figure 25. The control group showed a drop in mean weights for a period of four months but then recovered to previous levels. The mean body weight differences noted in the guinea pig groups were transient in nature and not consistent throughout the study. A dose-response relationship was not seen.

The objective of bracketing the effect threshold of fluomine for a six-month intermittent exposure was attained with the concentrations chosen. Certainly, the increased lung weights in rats exposed to 2 mg/m^3 fluomine for two weeks along with the mortality in guinea pigs demonstrated that this concentration would not be suitable for a six-month exposure. Analysis of all of the data obtained from exposed and control animals showed that exposure to 0.1 or 0.5 mg/m^3 fluomine intermittently for six months had little or no effect on mortality or gross pathology findings. The body weights of rats and guinea pigs were somewhat lower in exposed groups, but there was no correlation with dose.

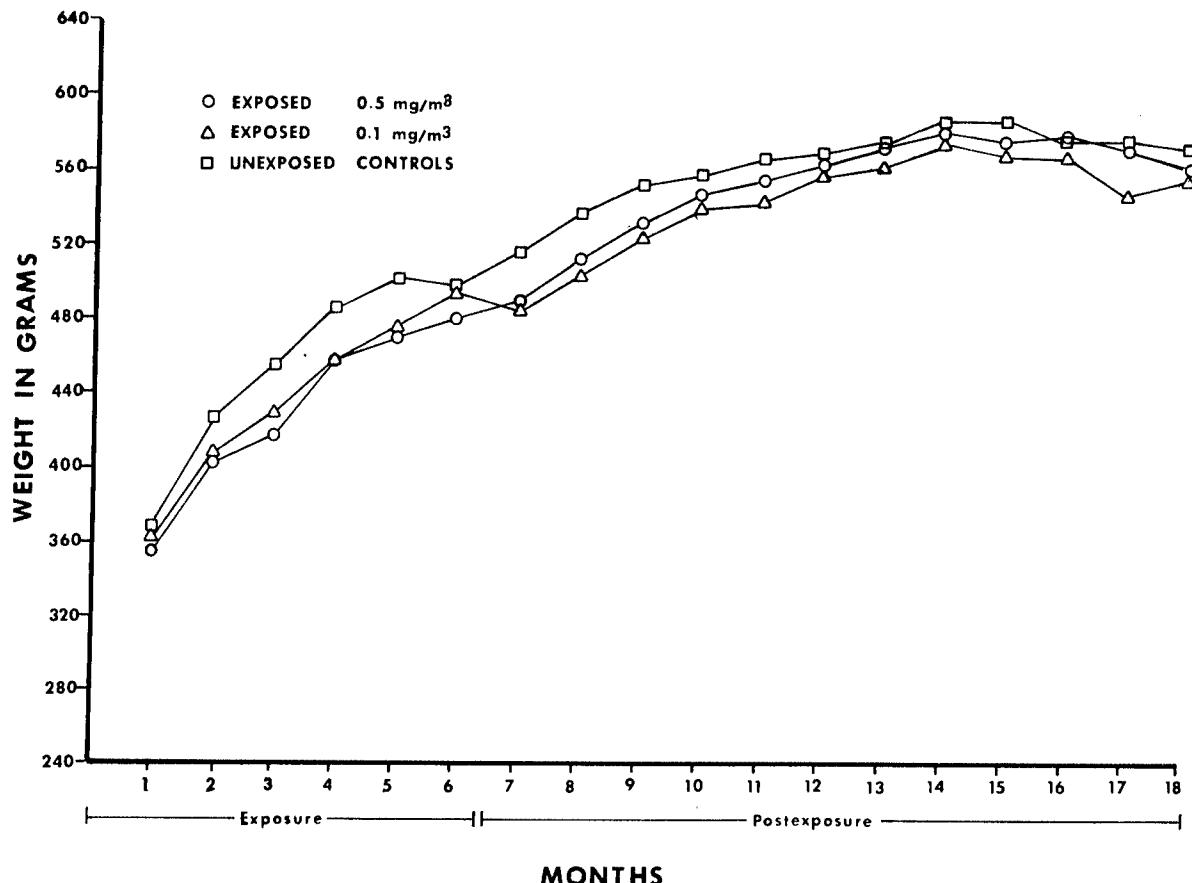


Figure 25. Effect of fluomine dust inhalation on guinea pig body weight.

Histopathologic examinations of animals sacrificed immediately postexposure showed that exposure to the higher dose had significant deleterious effects on the lungs and livers of dogs, while effects of the 0.1 mg/m^3 exposure in this species were absent or minimal. Rats in both the 0.1 mg/m^3 and the 0.5 mg/m^3 exposed groups showed about the same incidence of chronic inflammation of the trachea. Although there was no dose dependency of tracheitis incidence, bronchopneumonia during a 12-month postexposure period developed only in rats exposed to the higher level indicating that this condition is a delayed effect of exposure to fluomine. No other significant nononcogenic lesions were found in any species.

The only tumor which showed any increase in exposed animals was alveolar/bronchiolar adenoma in mice. There was an increased incidence of borderline significance in animals exposed at the higher level in the last six months of postexposure holding.

Challenging guinea pigs with intradermal injections of fluomine suspensions after 2 weeks exposure to 2 mg/m^3 or after six

months exposure to 0.5 mg/m³ demonstrated that no hypersensitization had occurred in the animals as a result of these exposures.

The concentration of 0.1 mg/m³ is close to the effect threshold of fluomine at or below which no deleterious sequelae may be expected. Care must be taken in extrapolating these results to man because of the highly irritating nature of the compound to eyes and lungs and because of its potent sensitizing properties.

EMERGENCY EXPOSURE LIMIT STUDIES WITH JP-10

JP-10 is a synthetic saturated polycyclic hydrocarbon which, because of its high density and other physical chemical properties, is utilized as a jet fuel either alone or as a major constituent (70%) of JP-9 jet fuel. In the latter application, it has been substituted for RJ-4, a reduced dimer of methylcyclopentadiene. Acute toxicity studies on this fuel were done by this laboratory and reported in a previous annual report (MacEwen and Vernot, 1979).

The purpose of this experiment was to establish emergency exposure limits for 60, 30, and 10 minutes. An emergency exposure limit is defined as that concentration which will not cause chronic or irreversible tissue damage or produce effects which would impair coordination and prevent a man from self rescue.

JP-10 is a single chemical entity identified as tricyclo (5.2.1.0^{2,6}) decane. Gas chromatographic analysis of samples from two drums of this material indicated that it was 98% pure JP-10 and 2% miscellaneous unidentified impurities. The material was supplied by the Air Force and was received from Suntech, Inc., Marcus Hook, Pennsylvania.

The physical properties of JP-10 are as follows:

Molecular weight	136
Boiling Point	360F
Density, 70F	0.940
Viscosity, 70F	3.5 CP
Flash point	135F

The methods for the generation of JP-10 vapor for exposure and the analytical monitoring techniques were described in a previous report (MacEwen and Vernot, 1979).

Groups consisting of 20 male Sprague-Dawley rats, 20 female ICR mice and 4 Beagle dogs were exposed to JP-10 vapor for one-hour periods. A control group of each species was maintained for

comparison to the test group. Ten animals of each rodent species, including controls, were killed immediately following exposure for examination while the remaining animals were observed for 28 days postexposure.

All animals were carefully observed for signs of toxic stress during and after the exposure period. The rats were weighed immediately prior to exposure, on the first, second, and third days postexposure, and weekly thereafter.

Prior to exposure, the beagle dogs were trained to perform four basic tasks. The dogs were trained to fetch, come, stay, and lead. It was necessary to spend considerable time acclimating the dogs to the laboratory environment. Approximately six weeks of training were done. The dogs were tested weekly prior to the exposure and twice immediately after the exposure.

Besides the field trial evaluation, the dogs were neurologically examined after exposure using the following tests: flexor reflex, extensor thrust reflex, tonic neck reflex, righting reflex, and placing reflex. Each reflex was tested by the method described by Hoerlein (1971). The dogs were weighed before exposure and at 2 and 4 weeks postexposure. On the same schedule, blood samples were taken on each dog for the following determinations:

HCT	SGPT	Sodium
HGB	SGOT	Potassium
RBC	Alkaline Phosphatase	Glucose
WBC	Bilirubin	BUN.

Gross and histopathologic examinations were done on all experimental and control animals from this study. All major organs were examined and sampled with special emphasis on the liver and kidney.

The first one-hour concentration tested, 150 ppm to both rats and mice, caused immediate hyperactivity in both species. Coordination of both species remained normal throughout the exposure.

The second one-hour exposure, to a mean concentration of 254 ppm, also caused hyperactivity in both animal species. After 51 minutes of exposure, one mouse had a tonoclonic spasm which lasted for 20 seconds, after which it appeared relatively normal. The convulsions of this animal would negate this concentration for consideration of an emergency exposure level.

No signs of stress were noted during the subsequent 28-day observation period in either species, and mean body weight gains of the test animals compared favorably with their respective controls. Gross and histopathologic examinations of the animals that were sacrificed immediately following exposure and those

sacrificed after 28 days revealed no lesions which could be attributed to the JP-10 exposure. Below is a detailed description of the rodent and dog exposure and assessment.

From the results of rodent exposures, a concentration of approximately 150 ppm was considered a safe one-hour exposure limit for rats and mice. The dog exposure was then designed for a nominal concentration of 150 ppm wherein the dogs would be carefully observed during exposure and tested postexposure for neurological effects.

The four test dogs were exposed to a mean measured concentration of 151 ppm for one hour. The dogs behaved normally throughout the exposure showing no signs of irritation or CNS effects. Immediately following the exposure, each dog performed the four trained tasks with its assigned animal trainer. All dogs performed this exercise adequately to the standard established during the training program. The subsequent neurological testing of each dog revealed no exposure-related effects. During the 28-day postexposure observation period, all dogs appeared normal. Blood samples examined at 14 and 28 days post-exposure showed all normal values. Gross and histopathology at necropsy revealed no exposure related lesions.

Because of an unavoidable delay in receiving additional rats, mice alone were exposed to the following 10-minute exposures. The first exposure, to a mean concentration of 1218 ppm, caused an increase in activity as seen in the previous exposures. No other observable symptoms were noted throughout the remaining exposure time or during the subsequent 28-day observation period.

The second exposure was to a mean concentration of 1211 ppm. During the 10-minute exposure period, the only sign noted was the usual increased activity of the mice. However, upon removal, one mouse had fine tremors and poor coordination while several other mice showed eye irritation and slight loss of coordination.

Gross and histopathology of the mice sacrificed immediately following these exposures and after 28 days did not show any lesions which could be attributed to the chemical insult. Mean weight gains of the test mice after 28 days compared favorably with the mean weight gains of the control mice.

Because coordination effects precluded the higher concentration, a single rat exposure was run at 1015 ppm. The only sign of stress noted was hyperactivity similar to that seen in the dog exposure. No gross or histopathological lesions were observed.

all previous rodent exposures. Mean body weight gains and pathology examinations failed to show any differences when compared to the respective control groups.

The trained dogs were exposed to a mean concentration of 1000 ppm for 10 minutes. By two minutes, all dogs showed signs of eye irritation although only one dog experienced lacrimation. Two dogs displayed fine tremors at the conclusion of the exposure. In general, all dogs appeared less active during exposure compared to the two previous dog exposures.

Following exposure, all dogs performed the learned tasks with their trainers. Reflexes, tested by the Air Force veterinarians, appeared to be normal while blood parameters examined at 14 and 28 days were all within normal limits. Gross and histopathology at necropsy revealed no exposure related lesions.

The lack of serious toxic signs as well as the fact that no significant histopathology was found at the lower concentrations tested would result in the following recommendations for short-term exposure limits. The concentrations and times are 150 ppm for 60 minutes, 600 ppm for 30 minutes, and 1000 ppm for 10 minutes. The selection of 600 ppm at 30 minutes is based on the slight lacrimation in the dogs and coughing experienced at 700 ppm. We believe a concentration of 600 ppm would eliminate this problem.

EVALUATION OF THE IRRITATION POTENTIAL OF SEVERAL HYDRAULIC FLUIDS

The Toxic Hazards Research Unit was requested to examine samples of several hydraulic fluids for the U. S. Navy to evaluate their potential for skin irritation. Two of the samples were the same formulation, one fresh from the barrel and one which had been in use in a hydraulic system and taken from the fluid accumulator. The other samples were unused material of different formulations from other manufacturers.

Since one of these hydraulic fluids had been associated with dermatitis in workmen handling it, the THRU was requested to investigate the dermal and ocular irritation potential of all four samples in albino rabbits. The sensitization potential of these materials was also evaluated.

Female New Zealand albino rabbits weighing approximately 5 pounds and male Hartley derived, albino guinea pigs weighing between 400 and 600 grams were purchased from Sweetwater Farm, Incorporated.

The following hydraulic fluids were supplied by the Navy Medical Research Institute/Toxicology Detachment, Wright-Patterson Air Force Base, Dayton, Ohio.

WGF-200D, Wyandotte Company (accumulator sample)
WGF-200D, Wyandotte Company (barrel sample)
Houghto-Safe 271, Houghton Company
Houghto-Safe 273, Houghton Company
Fyrquel-220

The primary skin irritation potential of the hydraulic fluids was measured by a patch test technique on intact and abraded skin areas of albino rabbits. Six rabbits were used for the evaluation of each fluid. The dorsal area of each rabbit was clipped free of hair 24 hours prior to administration of the compound, thus allowing irritation from the clipping process to heal. Equal numbers of exposures were made on the intact and abraded skin. Abrasions were minor incisions through the stratum corneum which were not deep enough to disturb the derma or to produce bleeding. The materials were applied in quantities of 0.5 milliliters. Each site was covered with a 1 x 1 inch piece of surgical gauze two layers thick followed by dental dam and a 4 x 4 inch piece of Elastoplast adhesive tape. The rabbits were then fitted with leather restraining collars to prevent disturbance of the patch area. After 24 hours, the collars, dental dam, and patches were removed. Any reaction resulting from the test materials was evaluated at this time and again at 72 hours after application using the method of Draize et al. (1959).

All but one of the 5 materials tested gave primary irritation test scores of zero and Fryquel-220 produced very mild erythema at 24 hours which was gone by 72 hours. These scores indicate that these hydraulic fluids are not primary skin irritants.

A 0.1 ml sample of each fluid was applied to one eye of each of six albino rabbits. The opposite eyes of three were untreated and served as controls. The opposite eyes of the other three rabbits were treated and washed after thirty seconds to evaluate wash effectiveness. Examinations for gross signs of eye irritation were made at 24, 48 and 72 hours following application. Scoring of irritative effects was according to the methods of Draize (1959) in which corneal, iris, and conjunctival effects are scored separately.

None of the hydraulic fluid samples tested caused any ocular irritation in the rabbits. No differences could be noticed when comparing the test eyes and/or the washed eyes with the respective control eyes at 24, 48 or 72 hours.

Groups of test animals consisted of 20 male albino guinea pigs. All fluids except Fryquel-220 were injected as 0.1% solutions in distilled water. Fryquel-220 was injected in peanut oil. Control animals injected with peanut oil were also tested. The sensitization tests were started on a Monday when the guinea pigs were weighed and closely clipped on the scapular areas. A volume of 0.05 ml. of a 0.1% dilution of the test material was administered into the upper right scapular area of each guinea pig. A similar control administration of distilled water was made into the upper left scapular area. Readings were taken 24 hours later and recorded.

Doses of 0.1 ml of the same dilutions (freshly prepared) were then injected into the clipped dorsal lumbo-sacral areas of the guinea pigs on the following Wednesday, Friday, Monday, etc., until seven doses had been administered. The guinea pigs were rested for three weeks (incubation period), weighed and given a challenge injection of 0.05 ml of the test material at the lower right scapular area. The left scapular area was again used for vehicle control tests. The reactions were read after 24 and 48 hours and recorded.

The grading system was designed so that the intensity of the skin reaction is represented by a proportionate numerical value and also that any reaction elicited by the vehicle is subtracted from the reaction elicited by the test material and vehicle combined.

The following table shows a summary of the results of the skin sensitization tests. Each group of guinea pigs originally consisted of twenty animals. However, two guinea pigs in the group treated with the Houghto-Safe 271 hydraulic fluid died of extraneous causes during the experiment and were not replaced. The response for that compound shown in the table, therefore, is based on 18 animals.

Compound	Numbers Showing Response		Sensitizing Potential	Mean Reaction Score (24 hrs)	Sensitizing Response
	24 hrs	48 hrs			
WGF-200D (accumulator)	13	13	Severe	93	Moderate
WGF-200D (barrel)	14	11	Severe	80	Moderate
Houghto-Safe 271	2	4	Slight	34	Mild
Houghto-Safe 273	0	0	None	--	None
Fryquel-220	4	5	None	39	None

The sample of WGF-200D taken from the accumulator caused reaction scores greater than 100 in five guinea pigs at both the 24 and 48 hour examinations. The same material taken from the barrel resulted in five guinea pigs having reaction scores greater than 100 at 24 hours while one of these exceeded 200 at 48 hours. The responses shown for the Houghto-Safe 271 were less than 50 at 24 hours and less than 100 at 48 hours.

The evaluation of the sensitization response to Fryquel was hindered somewhat by the reaction of the guinea pigs to the peanut oil vehicle. Peanut oil produces mild primary irritation when injected intradermally in guinea pigs. However, the Fryquel was highly soluble in peanut oil and other solvents in which Fryquel was found to be soluble produced necrotic skin lesions after intradermal injection.

Under the conditions of these tests, Fryquel-220 was determined not to be a primary skin or eye irritant. Sensitization test numerical values obtained for Fryquel were very low and indicated that the material had minimal sensitizing potential.

DETERMINATION OF THE DERMAL TOXICITY OF JP-10

JP-10 is a synthetic saturated polycyclic hydrocarbon which because of its high density and other properties, is being utilized as a jet fuel, either alone or as a major constituent (70%) of JP-9 fuel. In the latter application, it has been substituted for RJ-4, a reduced dimer of methylcyclopentadiene. A series of acute toxicity tests, including EEL's, oral, intraperitoneal, eye and skin irritation, inhalation LC₅₀'s and sensitization, were done in our laboratory and reported by MacEwen and Vernot (1979).

This dermal toxicity study completed the acute data for this compound for establishment of laboratory safety standards.

Three rabbits were dosed with 20 mg/kg. This is the highest dose used in the percutaneous test. No deaths occurred in any of the 3 rabbits during the following 14 days. The rabbits gained an average of 100 grams during the observation period, a gain which would be considered subnormal. The skin was not burned or irritated following exposure, and hair growth in the exposed area was normal. JP-10 may be considered essentially nontoxic by the dermal absorption route.

DETERMINATION OF THE ACUTE TOXICITY POTENTIAL OF NAVY ANTIFOULING FORMULATIONS

Cuprous oxide is one of the biocidal chemicals being used in various paint formulations for painting the exterior surfaces of ships. This compound is an effective antifouling agent against

organisms such as barnacles, tubeworms, algae, sponges, etc.

Previous tests on this material indicated a low order of toxicity (LD₅₀ of greater than 5 gm/kg) when administered orally. Application of the compound to intact and abraded skin produced no irritation. The acute dermal toxicity was found to be greater than 20 gm/kg while instillation of the compound into rabbit eyes caused positive eye irritation. The material sprayed for one hour into a chamber containing rats caused no mortality or any observable effects in the rats at necropsy.

Acute toxicity information is needed on five antifouling paint formulations which are to be submitted to the Environmental Protection Agency for Pesticide Product Registration. In addition to the five formulations, the technical material (cuprous oxide) found in four of the paint formulations is also being reexamined for acute toxic properties.

The acute tests being attempted on each material are as follows:

a. Oral LD ₅₀	d. Primary eye irritation
b. Dermal LD ₅₀	e. One-hour inhalation LC ₅₀
c. Primary skin irritation	

All test material has been supplied by the Navy. The six materials being tested are listed below:

1. Paint formulation 105
2. Paint formulation 121/63
3. Paint formulation 129/63
4. Paint formulation 1020A
5. Paint formulation 134
6. Cuprous oxide

Methods and procedures for cutaneous, oral, irritation, and inhalation testing were described in a previous annual report (MacEwen and Vernot, 1979). This report also included the results of the oral and skin irritation tests for each of the formulations.

Cutaneous testing of all materials has been completed with the following results:

<u>Formulation</u>	<u>LD₅₀ (95% C.L.)</u> <u>in gm/kg</u>	<u>Data Used to Calculate</u> <u>LD₅₀ in gm/kg</u> <u>(Mortality Response, N = 3)</u>
134	16.8 (5.5-51.5)	10 (0), 20 (2)
1020A	11.9 (3.9-36.4)	10 (1), 20 (3)
121/63	-	20 (1)
129/63	-	20 (0)
105	-	20 (1)
Cuprous oxide	-	20 (1)

The most toxic of these materials by the cutaneous route is formulation 1020A with an LD₅₀ of 11.9 gm/kg. This formulation is the only one containing tributyltin compounds instead of cuprous oxide as the active ingredient.

Formulation 1020A and 134 would both be considered toxic while the other four materials would be less than toxic by cutaneous application.

The six materials were tested for eye irritation with examinations being made at 24, 48 and 72 hours after application. Scoring or irritative effects was according to the methods of Draize (1959), in which corneal, iris and conjunctival effects are scored separately. A summary of the effects is seen in Table 56.

Three of the six compounds were found positive for eye irritation effects. Of those three, formulations 1020A and 129/63 are definite irritants while formulation 105 is borderline.

Formulation 121/63 caused no eye irritation in any of the six rabbits tested. Formula 134 produced redness of the conjunctivae in five of six rabbits at 24 hours and three of six at 48 hours. The cornea and iris remained clear in all six rabbits. Circumcorneal injection was seen in one of six rabbits dosed with cuprous oxide at 24 hours but not thereafter. The conjunctivae of all six rabbits showed slight injection which included discharge in four of the six at 24 hours. At 48 hours, only two showed slight injection while all were clear by 72 hours. According to the test standards, these effects are not great enough for positive eye irritant classification.

Formulation F-105 caused a considerable amount of chemosis and discharge at 24 hours in two of six rabbits. The eyelids of these two rabbits sealed shut, and natural drainage and cleansing could not take place. At 48 hours, the one rabbit's eye had only slight redness while the second rabbit's eye showed corneal opacity which involved approximately 50% of the cornea. At 72

TABLE 56. RABBIT EYE IRRITATION RESULTS AFTER APPLICATION OF ANTIFOULING PAINT FORMULATIONS

<u>Rabbit No.</u>	<u>Numerical Score</u>		
	<u>24 Hours</u>	<u>48 Hours</u>	<u>72 Hours</u>
FORMULATION 134			
F56	6	2	2
F58	2	2	0
F60	2	0	0
F62	4	2	0
F64	2	0	0
Z53	2	0	0
	\bar{x} 3.0	1.0	0.3
FORMULATION 1020A			
F38	a	a	a
F40	a	110	110
F42	a	110	110
F32	a	110	110
F34	a	a	110
F36	a	a	110
a Eyelids swollen preventing scoring.			
FORMULATION 121/63			
E96	0	0	0
F10	0	0	0
F02	0	0	0
F16	0	0	0
F18	0	0	0
F14	0	0	00
FORMULATION 129/63			
F28	65	59	51
F30	19	4	0
F20	17	8	4
F24	4	0	0
F26	11	0	0
F22	19	11	6
	\bar{x} 22.5	13.7	10.2
FORMULATION 105			
E98	13	4	0
F04	19	4	4
F00	4	2	0
E94	21	34	26
F08	2	0	0
F12	0	0	0
	\bar{x} 9.8	7.3	5.0
CUPROUS OXIDE			
F33	6	0	0
F46	2	0	0
F48	11	2	0
X01	4	2	0
Y01	2	0	0
Z01	6	0	0
	\bar{x} 5.2	0.7	0.0

hours, the results were similar. Of the four rabbits that had cleared the compound from the eye and did not have the lids sealed, three showed slight redness and chemosis at 24 and 48 hours but were clear by 72 hours.

The eyelids of one rabbit dosed with 129/63 sealed shut during the initial 24 hours and, as a result, a considerable amount of chemosis and discharge was noted at 24 hours. At 48 hours, this rabbit showed corneal opacity in about 20% of the cornea which took a fluorescein stain. No corneal drainage damage was seen in any of the other five rabbits. Circumcorneal injection was seen in five of the six rabbits at 24 hours, two of six at 48 hours, and one of six at 72 hours. Moderate to severe irritation of the conjunctivae was seen in four of six rabbits at 24 hours. This persisted in the one rabbit through 48 and 72 hours.

Paint formulation 1020A sealed all six rabbits eyes closed. The resultant swelling of the eyelids made it impossible to score the eyes at 24 hours. By 48 hours, three of the six could be scored while five could be scored at 72 hours. All scores at these times were maximum damage values. This paint causes excessive opaqueness of the cornea and extreme irritation to the iris and conjunctivae. This compound is a severe eye irritant.

The oral dosing results of the five paint formulations were tabulated in the last annual report; however, the oral toxicity of cuprous oxide was not included. This compound was dosed as a suspension in a 50% Carbowax 4000 solution. Brij 35 at 0.3% was used as a surfactant. Control animals were dosed at 20 gm/kg with the vehicle with no resultant toxic effects during the 14-day observation period. The results of the oral dosing as well as the LD₅₀ are shown below:

<u>Cuprous Oxide Dose, gm/kg</u>	<u>Mortality Ratio, N = 5</u>
20	4/5
10	3/5
5	0/5
LD ₅₀ = 10 (5.9 to 17.0) gm/kg.	

Acute inhalation testing has not been done due to problems involving developing a suitable generation technique. Various methods have been tried ranging from homemade aerosolizers to commercial paint sprayers with only minimal success. Because of the high density of the paint, a conventional sprayer is inefficient in producing fine particulates. The maximum respirable aerosol concentration which could be achieved using these generators was 25 mg/m³. We are now investigating the possibility of obtaining a high-pressure sprayer from one of the Navy depots for use in this study.

PERCUTANEOUS, ORAL, AND INHALATION STUDIES FOR
CLASSIFICATION OF TOXICITY RATINGS FOR
TRANSPORTABLE CHEMICAL AGENTS

Certain materials being transported have inadequate toxicology data which is necessary for proper classification by the Department of Transportation. These materials were tested in this laboratory to verify the suitability of proposed transportation health hazards classification criteria. This was done by determining experimentally the 14-day toxicity by skin absorption on rabbits, peroral LD₅₀'s, and one-hour inhalation LC₅₀'s to male and female rats.

The toxicity classification system published in a previous report by Back et al. (1972) was used to categorize the compounds in the present study. The following criteria were used to determine the category in which each compound was placed.

	<u>Extremely Toxic</u>	<u>Highly Toxic</u>	<u>Toxic</u>
Inhalation, 1-hour LC ₅₀	500 mg/m ³ or less (50 ppm or less)	>500-2000 mg/m ³ (>50-200 ppm)	>2000-200,000 mg/m ³ (>200-20,000 ppm)
Skin Absorption (Dermal) LD ₅₀	20 mg/kg or less	>20-200 mg/kg	200-20,000 mg/kg
Oral, 14-Day Single Dose LD ₅₀	5 mg/kg or less	>5-50 mg/kg	>50-5000 mg/kg

During the current reporting period, a group of compounds was received and assigned code numbers prior to testing. These compounds, their THRU code numbers and the tests to be done on each are listed in Table 57.

The three compounds on which inhalation studies were conducted are water soluble crystalline solids. Animal inhalation exposures were conducted in a 60-liter transparent plastic chamber to aerosols of the test agents generated from aqueous solutions.

The aerosol of selenium oxide was formed by aspirating a solution of SeO₂ into the air stream of an all glass nebulizer. The aerosol concentration in the chamber was controlled by varying the concentration of selenium dioxide in solution with distilled water while maintaining a constant chamber airflow rate of 15 liters/min. Chamber concentrations of SeO₂ were monitored

TABLE 57. LIST OF COMPOUNDS SUBMITTED BY THE
DEPARTMENT OF TRANSPORTATION FOR ACUTE INHALATION,
PERORAL AND PERCUTANEOUS TOXICITY STUDIES

<u>Code No.</u>	<u>Compound</u>	<u>Peroral</u>	<u>Inhalation</u>	<u>Skin Absorption</u>	<u>Skin Corrosion</u>
267	Oxalic Acid		X		
307	Selenium Oxide	X	X	X	
308	Vanadyl Sulfate	X	X	X	
309	Pentachlorophenol				X

continuously by sampling in a scrubber tower with 0.125 N KI in 0.2 N HCl and analyzing iodine formed with the flow colorimeter of a Technicon AutoAnalyzer II which was calibrated against standard solutions.

Vanadyl sulfate aerosol was generated in the same manner as selenium oxide and was also analyzed by air sampling with a glass scrubber tower. The sampling solution used was dilute sulfuric acid (pH 1.6). The sampling solution was passed through a flow cell of the Technicon AutoAnalyzer II and vanadyl sulfate concentration was measured on the colorimeter using a 600 nm filter. The sampling solution was used in the preparation of standards for calibration of the colorimeter.

Oxalic acid aerosol was generated from a saturated water solution using an ultrasonic nebulizer. The aerosol was generated in a transparent plastic plenum chamber and drawn into the animal exposure chamber. Continuous analysis of the oxalic acid aerosol concentration in the exposure chamber was determined in a manner similar to that used for vanadyl sulfate and selenium dioxide. The oxalic acid aerosol was collected in a buffered KI-KIO₃ solution in the scrubber tower and the iodine formed was measured colorimetrically.

The results of the completed acute toxicity tests and the assigned classifications are shown in Tables 58 through 61.

The one-hour aerosol inhalation results for oxalic acid and vanadyl sulfate show a sex-related difference in toxicity. In both cases, the female rats are less responsive to the toxic effects of the aerosol. Vanadyl sulfate appears to be equally toxic to both sexes by the peroral route.

TABLE 58. ORAL TOXICITY OF VARIOUS COMPOUNDS TO MALE AND FEMALE RATS

Compound	Sex	LD ₅₀ (95% C.L.) in mg/kg	Data Used to Calculate LD ₅₀ (N = 5)	Classification
Selenium Oxide	M	54 (36-79)	25 (0) ^a , 50 (2), 100 (5)	Highly Toxic
	F	31 (22-42)	12.5 (0), 25 (1), 50 (5)	Highly Toxic
Vanadyl Sulfate	M	493 (358-678)	200 (0), 400 (1), 800 (5)	Toxic
	F	566 (369-867)	200 (0), 400 (1), 800 (4), 1600 (5)	Toxic

^a Number of deaths.

TABLE 59. DERMAL TOXICITY OF VARIOUS COMPOUNDS TO FEMALE RABBITS

Compound		LD ₅₀ (95% C.L.) in mg/kg	Data Used to Calculate LD ₅₀ mg/kg (N = 5)	Classification
Selenium Oxide		100 (28-43)	78 (1) ^a , 156 (2), 313 (3)	Highly Toxic
Vanadyl Sulfate		4450 (No range)	3150 (0), 3970 (0), 5000 (3)	Toxic

^a Number of deaths.

TABLE 60. ONE-HOUR INHALATION TOXICITY OF VARIOUS COMPOUNDS FOR MALE AND FEMALE RATS

Compound	Sex	LD ₅₀ (95% C.L.) in mg/kg	Data Used to Calculate LD ₅₀ mg/m ³ (N = 5)	Classification
Selenium Oxide	M	100 (60-187)	50 (1) ^a , 97 (1), 109 (3), 208 (5)	Extremely Toxic
	F	103 (95-114)	69 (0), 94 (2), 96 (0), 103 (3), 110 (3), 123 (5)	Extremely Toxic
Oxalic Acid	M	4215 (2743-6476)	2500 (1), 3570 (3), 3590 (1), 4950 (3)	Toxic
	F	b	4030 (0), 4920 (0)	--
Vanadyl	M	1579 (1217-2220)	870 (0), 1170 (1), 1500 (3), 1750 (3), 2350 (4)	Highly Toxic
	F	2458 (1875-3574)	1850 (0), 2270 (1), 2660 (4)	Toxic

^a Number of deaths.

^b The highest aerosol concentration that could be generated resulted in no deaths in female rats.

TABLE 61. CORROSIVE EFFECT OF PENTACHLOROPHENOL ON
RABBIT SKIN

<u>Rabbit No.</u>	<u>Result</u>
1	Negative
2	Negative
3	Negative
4	Negative
5	Negative
6	Negative

Result: Noncorrosive

SECTION III

FACILITIES

The support activities of the THRU essential to the operation of a research activity are usually not of sufficient magnitude to merit separate technical reports. Therefore, these activities are grouped together under the general heading "Facilities" to describe their contributions to the overall program of the laboratory.

COMPUTER PROGRAM DEVELOPMENT

During the past year, a number of new computer programs were developed, and additions and alterations were made to a number of established programs. These are listed below along with the reasons for changing or creating the program:

1. Pathology accession numbers were added to the animal mortality data base. This was done retroactively for all current experiments and permits easy identification of records in THP where accession numbers are the primary means of animal coding.

2. A program of regression on the "One-Hit" model of oncogenesis was written to predict low dose risk estimates of tumor incidence from experimental data at high doses. This program finds the best estimate of the parameter, β , in the expression

$$Pr \alpha = 1 - EXP (-\beta D)$$

Where $Pr \alpha$ = Predicted Tumor Incidence

D = Dose

β = Experimental Parameter

Confidence limits for any dose are also calculated. This regression program is only one of a number of methods used for estimating low dose effects and should not be used alone since there is usually no way to test whether it really represents the actual tumor induction process.

3. A program was acquired from Dr. Kenneth Crump of Louisiana Technical University which calculates the best estimates of the parameters, q_0 , q , - - - q_k , in this dose response model:

$$Pr \alpha = 1 = EXP [-(q_0 + q, D - - - + q_k D^k)]$$

This is essentially a generalization of the "One-Hit" model but without its implications concerning the molecular basis of tumor induction. Essentially, it creates a family of curves to one of which almost any data set may be fitted for extrapolation purposes.

4. Computer generated graphs of clinical chemistry and body weight means have been standardized by two new programs. Using these, the ordinate or y-axis representing the measured data is divided to give unit scale points, and the range of values is made large enough to include all expected observations.

5. A management information system was developed for use on the in-house microprocessor. Its first application is for the Facility Engineering Department Work Request File. Using this system, the responsible engineer can determine the status of any project for which a work request has been filed. Further applications are projected in the areas of inventory, scheduling and preventive maintenance.

GAS CHROMATOGRAPHIC EVALUATION OF RJ-5

Gas chromatograms of RJ-5, a mixture of isomers of norbornane dimer, were received from the distiller supplying the fuel to the Air Force. These were obtained using a capillary column coated with OV-17, a silicone polymer. As a first step in developing a gas chromatographic (GC) procedure for quality control analysis of RJ-5, an attempt was made to duplicate the GC conditions of the supplier. The column coating used was OV-101, a silicone polymer with polarity similar to that of OV-17. The chromatographic conditions used for the separation of isomers in the THRU laboratory are shown in Table 62.

TABLE 62. GAS CHROMATOGRAPHIC CONDITIONS FOR
SEPARATION OF RJ-5

Varian 3700 GC

Injector:	250C, 10:1 split ratio
Detector:	250C, FID, full sensitivity
Column:	OV-101 WCOT fused (silica) 50 meter capillary column
Column Oven:	110° for 5 minutes then 2° C/minute to 180°C for 10 minutes
Carrier Gas:	He at 32 psi (approx. 0.5 ml/minute)
Integrator:	Atten. 2 ⁵
Sample:	1:1000 RJ-5 in acetone
Injection:	1 μ l

Figure 26 is a comparison of the chromatograms obtained from the supplier and the THRU laboratory. Separation of peaks is quite comparable in the two chromatograms, although resolution may be somewhat better in the curve from the supplier. However, the distributions of the concentrations of the various isomers are somewhat different in the two chromatographs implying different mixtures. This is reinforced in Table 63, which compares relative peak areas in the two curves and shows that peak 9 is much larger in the THRU chromatogram and peaks 1 and 7 in the chromatogram made by the distiller.

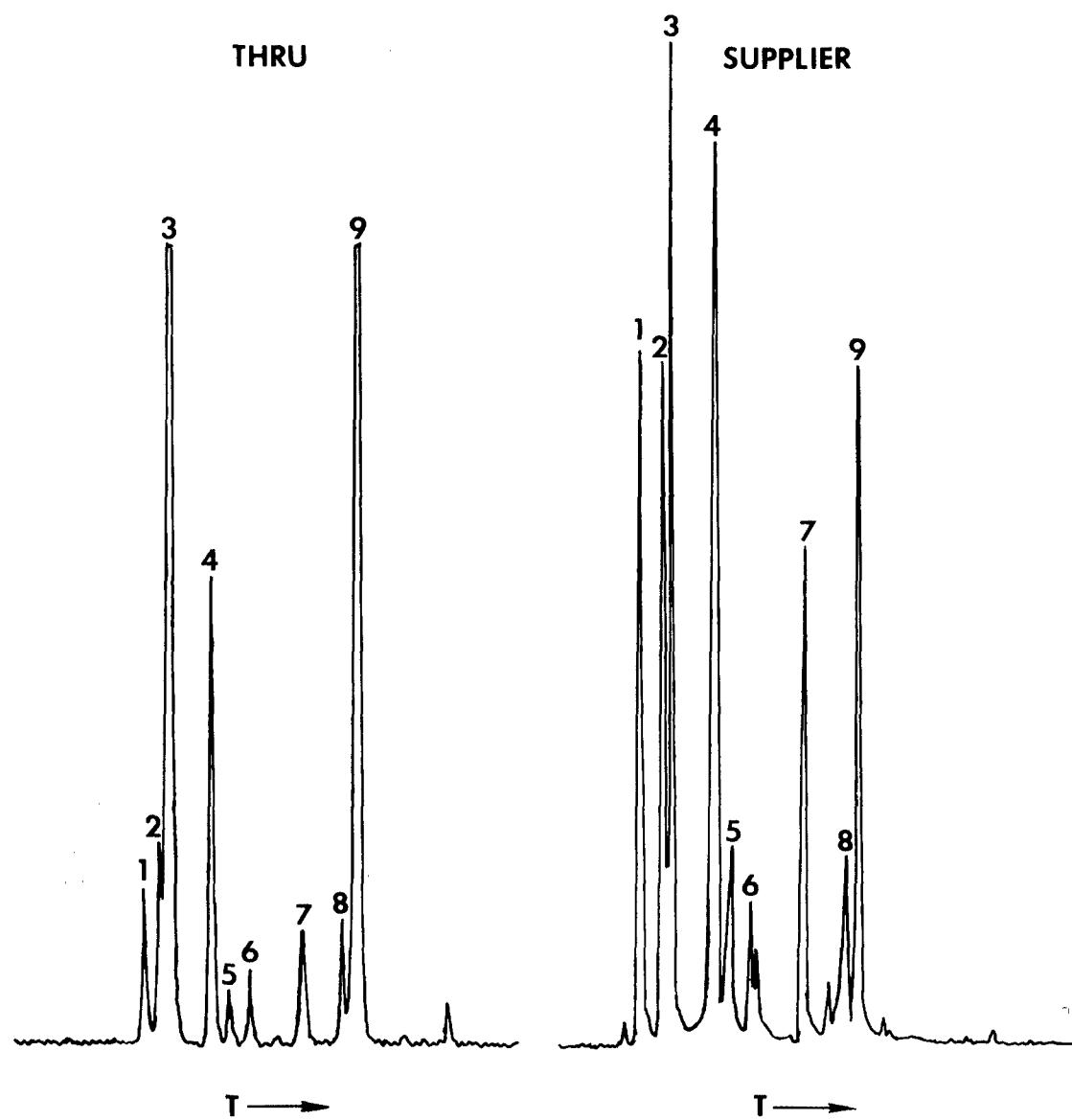


Figure 26. Comparison of gas chromatograms of RJ-5 run at the THRU and commercial supplier.

TABLE 63. COMPARISON OF RELATIVE PEAK AREAS IN GAS CHROMATOGRAMS OF RJ-5

Peak No. from Figure 26	Area Percent	
	THRU	Supplier
1	3.3	14.3
2	3.7	7.5
3	33.8	28.6
4	10.9	16.6
5	1.2	3.7
6	1.7	3.9
7	3.3	8.2
8	2.6	3.8
9	39.5	13.4

Using this technique also demonstrated that the mixture was stable in composition over daily use and over the time taken to consume one 55 gallon drum of material.

QUALITY CONTROL ANALYSIS FOR FYRQUEL-220

Fyrquel-220 is a hydraulic fluid composed of tri-aryl-phosphates which is manufactured by Stauffer Chemical Company and is being considered for use by the U. S. Navy. Quality control analyses were requested on this fluid to insure that the level of triortho-cresyl-phosphate (TOCP) was less than 1% as stated by the manufacturer. In order to accomplish this, a gas chromatographic analytical technique was developed to give maximum separation in the area where TOCP eluted from the analytical column. The chromatographic conditions which gave the desired separation are listed in Table 64.

TABLE 64. GAS CHROMATOGRAPHIC CONDITIONS FOR FYRQUEL-220 ANALYSIS

Varian 3700 GC

Detector: FID at 64 X 10-11 amps/mv at 290C
 Injector: On column at 240C
 Column: 1/8" X 10' SS, 10% SE-30 on Chromosorb W-HP
 Column Oven: 150C to 280C @ 6C/minute and hold
 Chart Speed: 1 cm/minute
 Sample: 0.5 μ l injection of 1/10 dilution of Fyrquel in hexane.

Chromatographic analysis of Fyrquel-220 showed a mixture containing at least 20 components as illustrated in Figure 27. Analysis of TOCP under the same chromatographic conditions showed three peaks with the largest accounting for almost 98% of the total area of the chromatogram and having a retention time of 23.42 minutes.

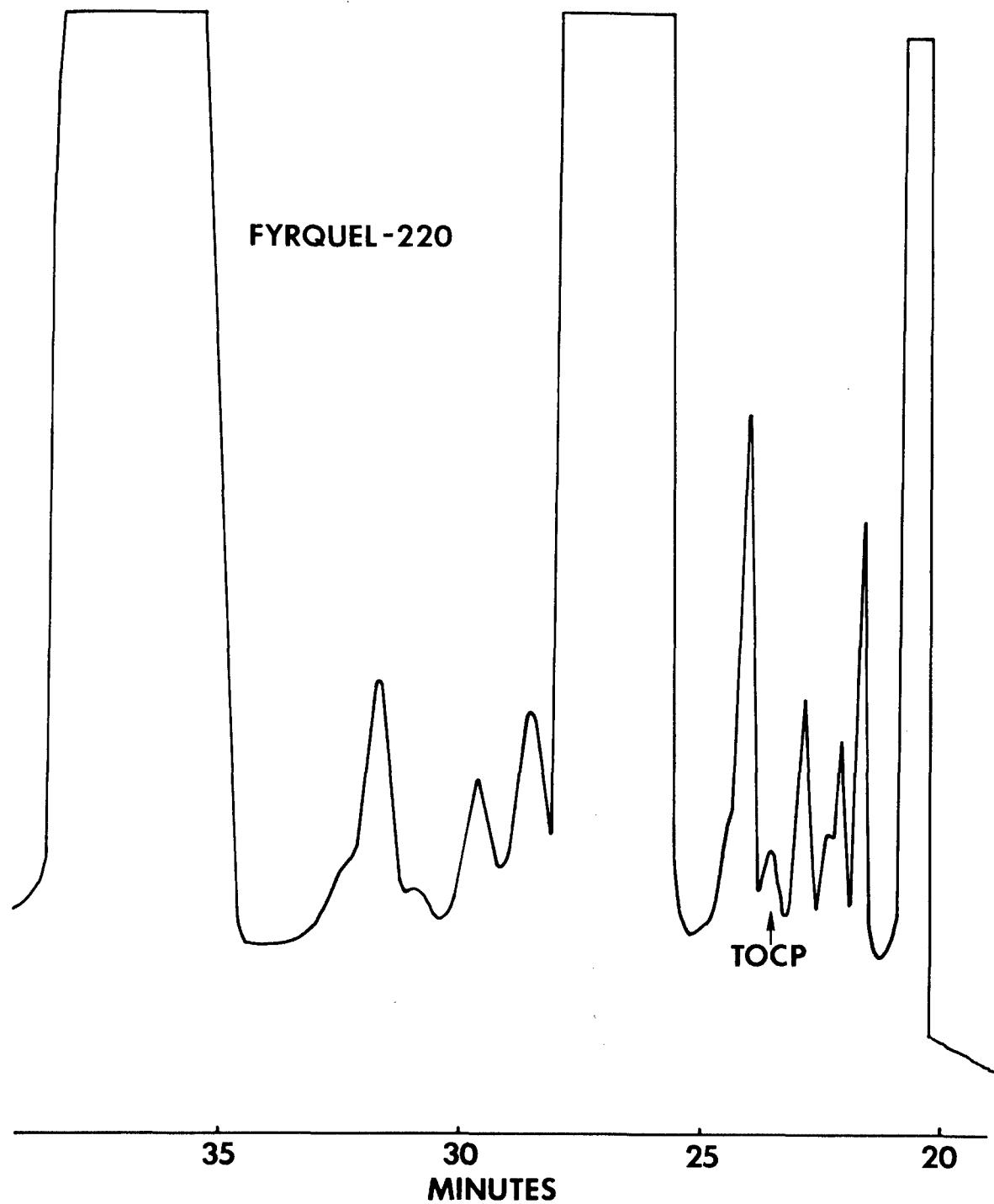


Figure 27. Gas chromatogram of Fyrquel-220.

This peak's retention time matched well with a small peak in the Fyrquel-220 chromatogram eluting at 23.45 minutes. In order to help confirm the presence or absence of TOCP in the Fyrquel-220, a sample spiked with 10% TOCP was analyzed under the same chromatographic conditions. The spiked sample showed a single,

larger peak at 23.43 minutes. Therefore, on the basis of GC analysis, the concentration of TOCP in Fyrquel-220 was calculated to be approximately 0.3%.

EVALUATION OF SHALE JP-5 CONTAMINATION WITH AVIATION GASOLINE

Initial exposure to shale oil JP-5 was begun using material from 5 drums which were sent from the storage tank at Rickenbacker Air Force Base some days before the main shipment of approximately 65 drums was delivered. Gas chromatographic analyses of samples from the first 5 drums indicated they were virtually identical and contained a significant amount of low-boiling materials including toluene. After delivery of the main shipment, 3 drums of it were received by the Chemistry Department of the Toxic Hazards Research Unit for use in the exposure. Gas chromatographic analyses of these drums revealed that the material in them differed significantly from that seen in the first 5 drums. The fuel in the latter 3 drums contained hardly any of the low-boiling compounds seen in the earlier samples.

When this information was given to the Toxicology Detachment of the Naval Medical Research Institute, they analyzed each of the remaining drums and determined that only 4 contained the low-boiling compounds found in the first 5 drums received. Because of the uncertain composition of the material used in the study, it was terminated after 60 days exposure to dogs and mice.

Investigation revealed that the tank in which the shale JP-5 had been held prior to shipping to Wright-Patterson Air Force Base had previously held aviation gasoline. The Toxicology Detachment of the Naval Medical Research Institute obtained a sample of the aviation gasoline (grade 100/130) which had been held in the storage tank. This sample was analyzed gas chromatographically and found to resemble closely the groups of low-boiling compounds seen at the start of the chromatogram of the material used in the animal exposures. Figure 28 is the chromatogram of the aviation gasoline; Figure 29 is a typical chromatogram of the material used for exposure; and Figure 30 is a chromatogram representative of the bulk of the fuel delivered which does not contain a significant amount of low-boiling compounds.

The chromatogram in Figure 29 can be constructed by combining those in Figure 28 and 30 in the proper ratio. In order to confirm that a mixture of aviation gasoline and shale oil JP-5 would provide such a chromatogram, a mixture of 3% aviation gasoline and 97% shale JP-5 (which did not contain a significant

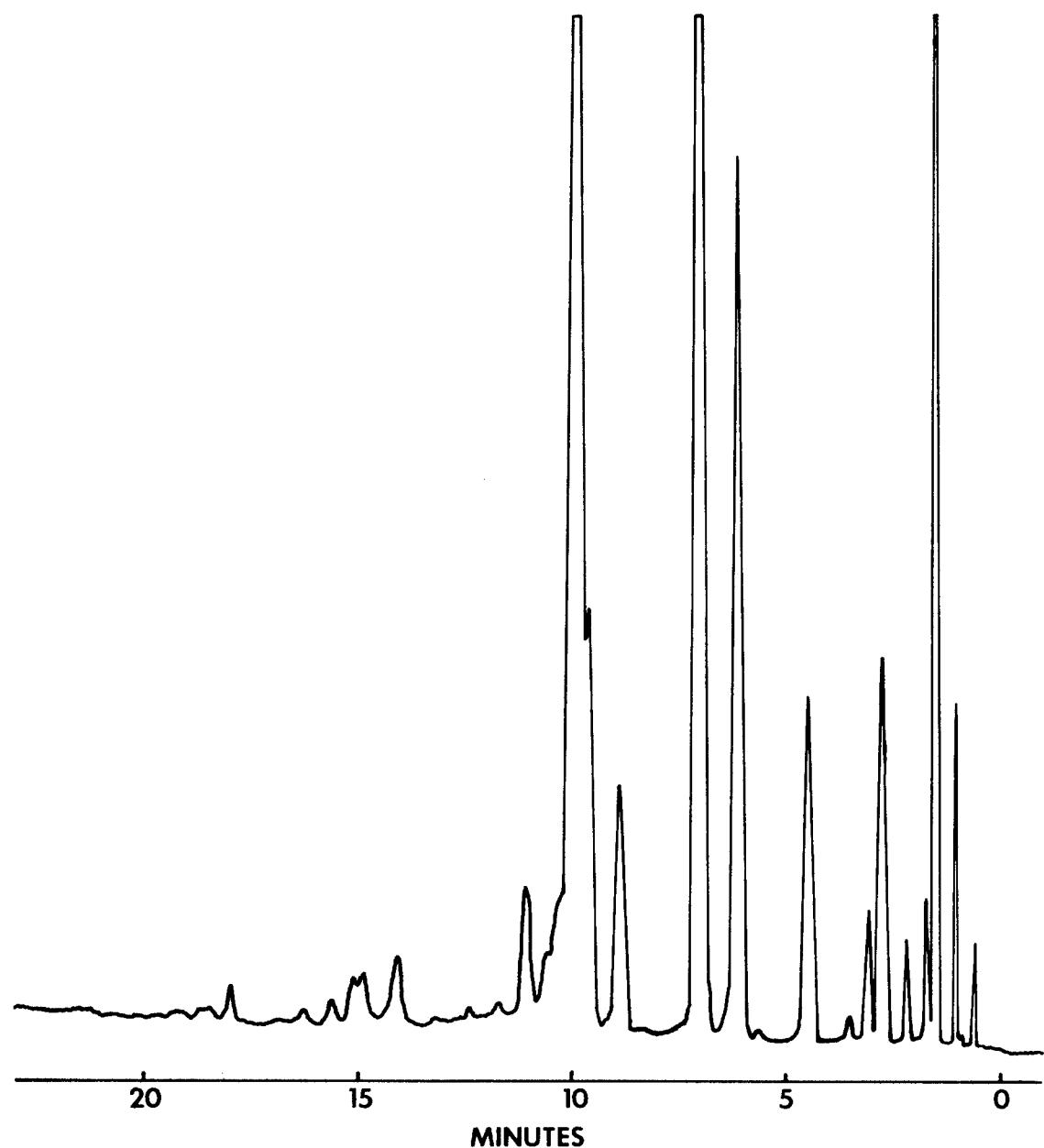


Figure 28. Gas chromatogram of aviation gasoline (grade 100/130).

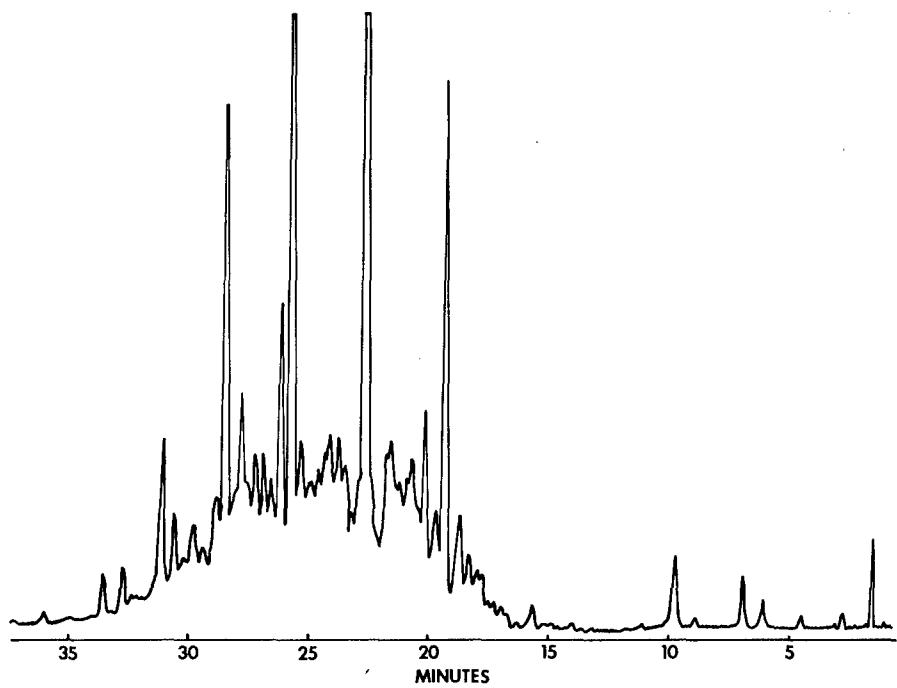


Figure 29. Gas chromatogram of liquid fuel used in chamber exposures contaminant.

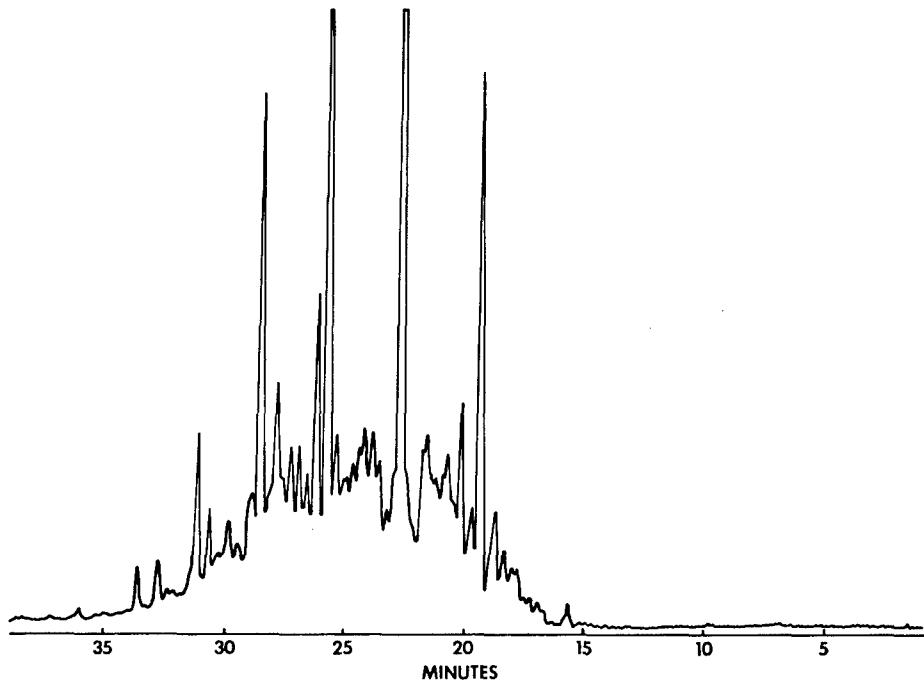


Figure 30. Gas chromatogram of uncontaminated liquid JP-5 shale fuel.

portion of low-boiling compounds) was made. The mixture gave the chromatogram shown in Figure 31 which is very similar to Figure 29 representing the material used in the exposure. The conclusions which may be drawn from these results are:

1. The contaminant in the 11 drums used for this exposure appears to have been aviation gasoline (grade 100/130).
2. The concentration of the aviation gasoline in the contaminated drums was between 3% and 5%. However, because of its high volatility, the contaminant represented more than 30% of the fuel vapors introduced into the exposure chambers.
3. There was shale JP-5 available which was not significantly contaminated by aviation gasoline and enough of this material was on hand to conduct a subsequent exposure.

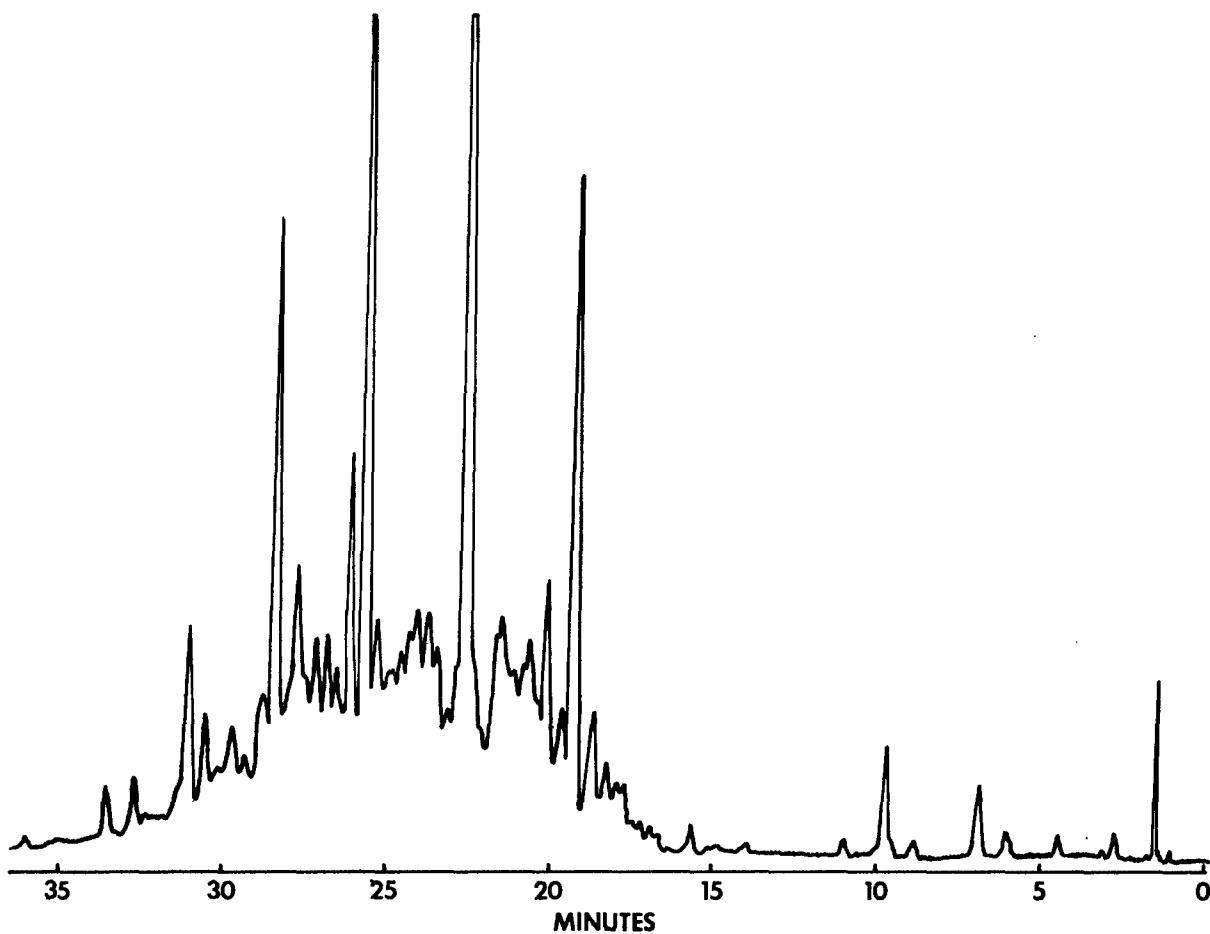


Figure 31. Gas chromatogram of 3% aviation gasoline (grade 100/130) in uncontaminated shale oil JP-5.

PHYSIOLOGICAL FLUIDS - DETERMINATION OF METHYLCYCLOHEXANE METABOLITES BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY

Results of a GC investigation into the metabolic products of inhaled MCH were given in the last annual report (MacEwen and Vernot, 1979). The authors noted that good separation of urine volatiles had been achieved by use of a capillary column coated with Carbowax 20M. A number of peaks not seen in control rat urine were present in the urine of exposed rats. These peaks had retention times which matched those of the isomeric methylcyclohexanols.

With the acquisition and installation of the Hewlett-Packard 5993A gas chromatograph/mass spectrometer (GC/MS) system, the GC conditions developed in 1979 were adapted to the GC/MS. Urine was sampled from rats being exposed to 400 ppm MCH on an industrial schedule by overnight collection. Gas chromatographic conditions were as follows:

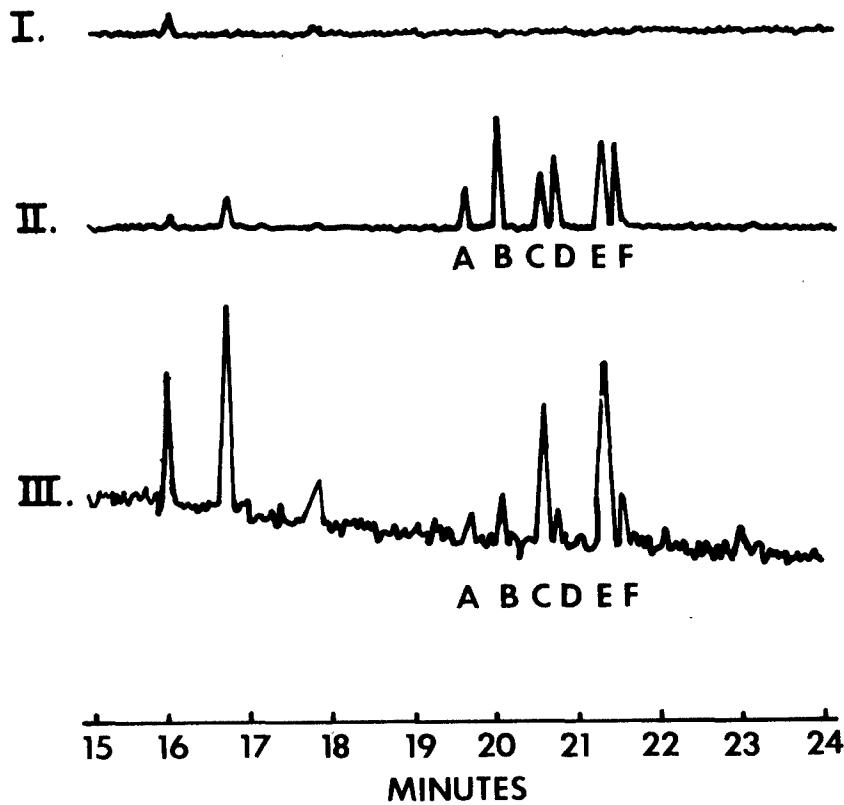
Column: 50 mx 0.25 mm WCOT, coated with Carbowax 20M
Carrier: He, 1.0 ml/min
Injector Temperature: 210C

Five microliters of untreated urine were injected. The nonvolatile portion was retained in the injector sleeve while the volatiles proceeded to the column. It was necessary to clean the injector sleeve after each injection since the residue acted to absorb and slowly release volatiles from subsequent injections. In order to check the cleanliness of the injector, distilled water samples were injected before urine samples.

Following this regimen, urine samples from control rats and those exposed to 400 ppm MCH were injected into the GC/MS. The total ion chromatograms of these samples are compared in Figure 32 where peaks are present in the chromatogram of urine from test rats in the retention region of 15-22 minutes. These peaks are absent from control rat urine. The 2, 3 and 4 methylcyclohexanols were chromatographed individually and as a mixture containing equal concentrations of each alcohol. Two peaks were present for each compound, indicating the presence of cis and trans isomers. Mass spectra were obtained for all peaks and compared with the spectra shown by the unknown peaks in urine from exposed rats. The retention time and spectrum of each isomer was compared with those of the corresponding peak in the urine GC, and good correspondence was found, providing strong evidence that the unknown peaks were, in fact, positional and geometric isomers of methylcyclohexanol. Shown in Figure 32 are gas chromatograms of urine samples in the limited retention time range from 15 to 24 minutes. Chromatograms represent samples of

LEGENDS

- I. Control rat urine.
- II. Urine from 400 ppm exposed rats with added isomeric methylcyclohexanols. (Attenuated)
- III. Exposed rat urine as is.



- A. Cis-2-methylcyclohexanol
- B. Trans-2-methylcyclohexanol
- C. Cis-3-methylcyclohexanol
- D. Cis-4-methylcyclohexanol
- E. Trans-3-methylcyclohexanol
- F. Trans-4-methylcyclohexanol

Figure 32. Partial gas chromatograms of control and MCH exposed rat urine with and without 50 ppm of each isomeric methylcyclohexanol added.

control rat urine, urine from rats exposed to 400 MCH as is and to which 50 mg/liter of each ring-substitution isomer has been added. The letters in the figure identify the added compounds associated with the peaks in the chromatograms for which mass spectra were obtained and which match the peaks in the urine from exposed rats. On the basis of GC retention times and mass spectra, the 2, 3, and 4 isomers of methylcyclohexanol have been identified in the urine of rats exposed to MCH. No evidence of the presence of any methylcyclohexanone was obtained, but a large peak was present at 16.8 minutes retention time in the GC of urine from exposed rats which has not yet been identified although it appears to have a parent peak at the same mass number as methylcyclohexanol. Since the only isomer of methylcyclohexanol which has not yet been obtained is the 1-substituted compound, this material will be acquired for comparison with the unknown material.

CHAMBER TECHNICIAN TRAINING PROGRAM

Since the last annual report, two technicians have been hired. Both technicians have completed the inhalation chamber operation training program described in last year's annual report as well as the Purina Animal Care self-study course. One of the newly hired technicians was certified by AALAS as an assistant animal technician and has had formal training in laboratory animal care. The other technician has had a number of years of experience in the animal care field and is qualified to take the examination for AALAS certification as a Laboratory Animal Technician. The new technicians also received training in blood sampling methodology and animal restraint.

Simulated emergency training procedures were conducted by the chamber technicians during the year. The situations serve as refresher training for the personnel involved. The procedures were conducted on each shift under the direction of a senior chamber technician with animal technicians or engineering technicians participating. A list of the various training procedures is shown below.

<u>Date</u>	<u>Procedure</u>	<u>Personnel Participation</u>
May 1979	Vacuum Pump Failure	A11
June 1979	Air Supply Fan Failure	A11
July 1979	Fire in Airlock During Entry	A,B,C
August 1979	Fire in the Exposure Laboratory	A11
September 1979	Complete Power Failure	A11
October 1979	Operation of Scott Air Pak	A11
November 1979	Complete Power Failure	A,B
December 1979	Rescue of Incapacitated Dome Entrant	A,B,C
January 1980	Fire in Airlock	A,B,C
February 1980	Operation of Scott Air Pak	A11
March 1980	Fire in Dome During Entry	A,B,C
April 1980	Air Compressor Failure	A11

A - Observer A

B - Observer B

C - Dome Entrant

All - All Chamber Technicians

ANIMAL TECHNICIAN TRAINING PROGRAM

Since last year's annual report, more technicians have become certified in the AALAS program. Two became certified at the highest level, Laboratory Animal Technologist, and two became certified at the first level, Assistant Laboratory Animal Technician. UCI animal care personnel certification in the AALAS program at present is as follows:

- 3 - Laboratory Animal Technologists.
- 3 - Laboratory Animal Technicians.
- 5 - Assistant Animal Technicians.

The basic course outline of certification by AALAS was described in detail in a previous annual report (MacEwen and Vernot, 1975).

An audiotutorial course on Laboratory Animal Medicine was utilized in our training program this year for animal technicians. The course was made available to UCI personnel by the Air Force Veterinary Medicine Division and the slide and cassettes were presented over a period of one month. A staff veterinarian was present or available while the program was presented to allow for discussion or questions following each session. Objectives for each section were distributed to each individual for self-examination. Upon completion of the program, quizzes were prepared and given from the objective list to stimulate interest and learning.

This course was a valuable training aid for the technicians in their daily routine as well as preparing for AALAS examinations. The following outline lists the titles which were covered in the training program.

LABORATORY ANIMAL MEDICINE AND SCIENCE AUDIOTUTORIAL SERIES

I. INTRODUCTION

- A. Laboratory Animal Medicine: What It Is and How it Relates to Veterinary Medicine
- B. Diseases of Laboratory Animals as Complications of Biomedical Research
- C. Legislation and Guidelines Pertaining to Laboratory Animals

II. THE MOUSE

- A. Biology and Use in Research
- B. Handling, Restraining and Other Techniques
- C. Husbandry
- D. Viral Diseases
- E. Bacterial and Parasitic Diseases
- F. Neoplastic, Non-Infectious and Miscellaneous Diseases

III. THE RAT

- A. Introduction
- B. Biology and Care
- C. Diseases and Their Control

IV. THE HAMSTER

- A. Introduction and Husbandry
- B. Biology
- C. Diseases

V. THE GUINEA PIG

- A. Introduction and Husbandry
- B. Biology
- C. Diseases

VI. NONHUMAN PRIMATES

- A. Taxonomy
- B. Biology and Use in Research
- C. Husbandry and Breeding
- D. Viral Diseases
- E. Bacterial Diseases
- F. Parasitic and Noninfectious Diseases

VII. AMPHIBIANS

 A. Medicine and Husbandry

VIII. REPTILES

 A. The Principal Bacterial Diseases of Captive Reptiles

IX. THE DOG AND CAT IN RESEARCH

 A. The Dog and Cat in Research

X. GNOTOBIOLOGY

 A. Gnotobiotics in Production of Experimental Animals

XI. THE RABBIT

 A. Introduction and Biology

 B. Husbandry and Techniques

 C. Pasteurellosis

 D. Parasitic Diseases

 E. Miscellaneous Disease

XII. OTHER INSTRUCTION

 A. Analgesics, Hypnotics, Sedatives and Anesthetics
 Used in Laboratory Animals

 B. Prevention and Control of Laboratory Animal Disease

Additional training in chamber technician duties was given to animal technicians to allow for their assistance when working weekends or evening shifts at the exposure facility. The third shift animal technician is now qualified to perform all chamber technician activities. The animal technician training includes the following chamber technician duties:

1. Scott Air Pak
2. Dome Entrant
3. Observer B
4. Chamber Technician
5. Emergency Procedures

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